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VIRUSES ASSOCIATED WITH CABBAGE MOSAIC [1]

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INTRODUCTION

A mosaic disease of cabbage (*Brassica oleracea* var. *capitata* L.) in southeastern Wisconsin was described by Larson and Walker in 1939.[3] The host range and certain properties of the infectious virus also were detailed. In 1937 Tompkins [4] gave an account of a mosaic disease of cauliflower (*B. oleracea* var. *botrytis* L.) in California, and in 1938 Tompkins, Gardner, and Thomas [5] described black ring, a virus disease of cabbage in the same State. In 1941 Larson and Walker [6] described another virus disease of cabbage, ring necrosis, in Wisconsin.

Further studies of the cabbage mosaic described earlier by Larson and Walker [3] have revealed that, as a rule, two distinct viruses (referred to herein as viruses A and B) occur systemically in infected plants. The present paper is an account of the separation of the two viruses, their properties, and the symptoms which they produce on various hosts independently and in combination. The cabbage black ring virus and the cauliflower mosaic virus of Tompkins [4] were studied comparatively in the same environment.

MATERIALS AND METHODS

The studies of symptoms, host range, and physical properties of the viruses were carried out in greenhouses which were fumigated frequently to keep them free from insects. Stock cultures of the viruses were carried in plants growing in specially caged insectproof compartments. Plants used for experiments were grown in 4-inch clay pots at temperatures suitable for their normal development. Mechanical inoculations with juice extracted from stock-virus plants were made by rubbing with cotton or cheesecloth over the surface of one or two lower leaves on which finely powdered carborundum had been sprinkled previously. Aphid transmission was carried out by transferring adult insects from virus-free nurture cabbage plants to the stock-virus plant and allowing them to feed for 48 hours or more. Aphids from the infected plant were transferred to the test plant by means of a camel's

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[2] The writers express sincere appreciation to Eugene H. Herrling for making the photographs used in this paper.

[3] LARSON, R. H., and WALKER, J. C. A MOSAIC DISEASE OF CABBAGE. Jour. Agr. Res. 59: 367–392, illus. 1939.

[4] TOMPKINS, C. M. A TRANSMISSIBLE MOSAIC DISEASE OF CAULIFLOWER. Jour. Agr. Res. 55: 33–46, illus. 1937.

[5] TOMPKINS, C. M., GARDNER, M. W., and THOMAS, H. R. BLACK RING, A VIRUS DISEASE OF CABBAGE AND OTHER CRUCIFERS. Jour. Agr. Res. 57: 929–943, illus. 1938.

[6] LARSON, R. H., and WALKER, J. C. RING NECROSIS OF CABBAGE. Jour. Agr. Res. 62: 475–491, illus. 1941.

hair brush, contact between the brush and the test plant being avoided. Sometimes a leaf with aphids was detached from the stock plant and placed on a small piece of paper and the paper was allowed to rest on a leaf of the test plant until the aphids had migrated to the new plant.

Upon the appearance of symptoms in a given test plant, the presence of the virus concerned was confirmed by mechanical transfer to cabbage or to tobacco (*Nicotiana tabacum* L.) when the latter was known to give a characteristic symptom. In the case of hosts within the Chenopodiaceae and Amaranthaceae it was learned that some constituent of the extracted juice of the host inactivated the virus and the presence of the virus was thus obscured. In such instances recovery was made only by means of insect vectors.

Physical properties of the viruses were studied with extracts from cabbage plants (var. Jersey Queen) infected with the virus or viruses in question. Infected plants were triturated with a sterile mortar and pestle. The crude extract was filtered through 8 thicknesses of cheesecloth without any application of pressure. For tests of tolerance to dilution the original extract was diluted with 9 parts of sterile distilled water in the case of virus B and with 99 parts in the case of virus A. This mixture was used in the preparation of each higher dilution tested. Dilutions were made in quantities of 100 cc. and shaken vigorously for 2 to 3 minutes, when a 10-cc. aliquot was drawn for the inoculation. A single pipette was used for each transfer; the inoculation from the highest dilution was made first, and successively lower dilutions were taken in order. For thermal inactivation determinations the filtered, undiluted extract was drawn into thin-walled, 2-mm. glass tubes which were sealed in a flame at one end and plugged with cotton at the other. The tubes were submerged in a circulating water bath for a 10-minute period at the desired temperature. The temperature was kept constant within ± 0.1°C. At the end of the period the sealed end of the tube was broken and the heated extract forced into a sterile mortar from which the inoculation was made. Determination of longevity in vitro was made by storing the filtered, undiluted extract in test tubes plugged with cotton in a constant-temperature incubator at 20°. A single test tube was removed at each stipulated interval and the contents used as inoculum without dilution. All glassware was sterilized before use. For cabbage virus A and the black ring virus, tobacco plants (var. Connecticut Havana No. 38) were used as test plants and determinations were made by counting the lesions on 2 leaves of each of 10 plants in each test. The same method was used for determining virus A in a mixture containing viruses A and B. For cabbage virus B and the cauliflower mosaic virus, 10 cabbage plants (var. Jersey Queen) per test were used.

Stock cultures of cauliflower mosaic virus and black ring virus were obtained from C. M. Tompkins, of the California Agricultural Experiment Station. The viruses of cabbage mosaic were obtained in southeastern Wisconsin. Certain comparative isolates [7] were collected from other parts of the United States.

SEPARATION OF THE TWO CABBAGE VIRUSES

In the original study of cabbage mosaic [8] it was found that the infectious entity was readily transferred to a wide host range. On

[7] The term "isolate" is used in this paper to designate a virus from a given source without implying that it is necessarily a distinct strain.

[8] See footnote 3.

tobacco systemic infection did not occur but local necrotic lesions up to 3 mm. in diameter were produced. In determinations of longevity in vitro, tolerance to dilution, and the point of thermal inactivation the local-lesion method of determining the activity of the virus extract was used. The thermal inactivation point determined by this method was 55° C. In later studies it was found that when the virus extract was heated several degrees higher than 55° it was still infectious on cabbage. Moreover, on cabbage plants so infected, symptoms differed from those which developed after inoculation with the original extract. This virus, hereafter referred to in this paper as cabbage virus B, produced symptoms not unlike an isolate obtained in the course of the earlier investigation by Larson and Walker [8] and set aside for further study.

Virus B was found to affect all cruciferous species earlier found susceptible to cabbage mosaic [8] except hoary alyssum (*Berteroa incana* (L.) DC.) and dames violet (*Hesperis matronalis* L.). It failed, however, to produce symptoms on any noncruciferous plant which had previously been found to react to the cabbage mosaic extract.

TABLE 1.—*Properties of cabbage virus A and black ring virus as determined by the number of local lesions formed on inoculated tobacco*

[2 leaves on each of 10 plants inoculated in each trial]

Type and degree of treatment	Lesions with—					
	Cabbage virus A				Cabbage viruses A and B	Black ring virus
	First trial	Second trial	Third trial	Fourth trial		
Dilution:	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
0	2,176	2,151	1,150	4,330	2,366	5,260
1-100	472	33	205	1,749	592	3,401
1-1,000	50	14	20	114	150	278
1-1,500	20	17	11	47	65	190
1-2,000	6	13	1	31	18	57
1-2,500	12	15	2	26	14	74
1-3,000	2	21	8	24	11	61
1-4,000	0	2	0	18	9	46
1-5,000	0	0	3	17	1	48
1-10,000	0	0	0	0	0	6
1-15,000				0	0	0
Heating for 10 minutes (° C.):						
None	421	2,151	494	501	2,128	2,458
51	232	1,111	139	412	776	1,620
52	209	917	111	350	314	1,350
53	183	896	87	279	222	250
54	180	681	72	179	180	144
55	170	486	29	86	58	158
56	60	468	3	44	20	28
57	18	65	3	7	18	24
58	7	19	1	1	26	2
59	0	6	0	1	4	0
60	0	0	0	0	0	0
61	0	0	0	0	0	0
Aging in vitro at 20° C.:						
None	2,842	3,110	2,430		2,260	3,990
1 day	2,460	2,980	1,760		2,012	3,484
2 days	1,606	1,700	136		1,050	2,905
3 days	580	538	8		202	314
4 days	468	416	3		17	54
5 days	472	286	6		4	4
6 days	406	136	7		10	2
7 days	164	0	4		4	0
8 days	0	0	0		0	0
9 days	0	0	0		0	0
10 days	0	0	0		0	0

[8] See footnote 3.

When noncruciferous plants were inoculated with a cabbage mosaic extract symptoms previously described [8] developed. However, when extracts from such plants were inoculated into cabbage, symptoms on the latter differed in certain respects, the virus thus obtained being free from cabbage virus B. The former is referred to hereafter in this paper as cabbage virus A.

PROPERTIES OF CABBAGE VIRUSES A AND B

When viruses A and B had been separated by the method described above they were carried in stock-virus plants for further study. Virus A was carried in plants of *Nicotiana glutinosa* L. The fact that this

TABLE 2.—*Properties of cabbage virus B and cauliflower mosaic virus as determined by the number of inoculated cabbage plants that became infected*

[10 plants inoculated in each trial]

Type and degree of treatment	Plants infected with—				
	Cabbage virus B				Cauliflower mosaic virus
	First trial	Second trial	Third trial	Fourth trial	
Dilution:	*Number*	*Number*	*Number*	*Number*	*Number*
0	10	10	10	10	10
1–10	9	6	7	10	10
1–50	6	4	4	8	9
1–100	5	1	3	5	8
1–500	2	0	0	1	5
1–1,000	2	0	0	1	2
1–1,500	0	0	0	0	1
1–2,000	0	0	0	0	0
1–3,000				0	0
Heating for 10 minutes (° C.):					
None	9	10	10	10	10
56	7	9	2	7	8
58	5	8	2	9	10
60	3	6	4	7	8
62	3	8	2	9	7
64	4	7	2	9	9
66	4	6	7	9	7
68	3	5	2	7	6
70	0	7	5	6	5
72	1	4	2	6	6
74	0	2	1	3	4
76	0	0	0	1	2
78	0	0	0	0	0
Aging in vitro at 20° C.:					
None	10	10	10	9	10
1 day	7	8	9	4	10
2 days	3	4	8	6	9
3 days	1	2	5	6	10
4 days	0	1	2	2	8
5 days	0	0	1	1	7
6 days	0	0	0	0	6
7 days	0	0	0	0	3
8 days	0	0	0	0	3
9 days	0	0	0	0	2
10 days	0	0	0	0	1
11 days	0	0	0	0	0
12 days	0	0	0	0	0
13 days	0	0	0	0	0
14 days	0	0	0	0	0
15 days	0	0	0	0	0

host was immune to virus B insured against chance contamination by the latter. Virus B was carried in cabbage plants. In order to insure against chance contamination with virus A, the extract was frequently

[8] See footnote 3.

heated to 65° C. before being transferred to new stock-virus plants.

The thermal inactivation points, the tolerance to dilution, and the longevity in vitro of the two viruses were determined by methods already described. The results are given in tables 1 and 2.

Virus A was inactivated by dilution at 1–4,000 in one trial, at 1–5,000 in one trial, and at 1–10,000 in two trials. Virus B was inactivated at 1–500 in two trials and 1–1,500 in two trials. Thus virus A was distinctly the more tolerant to dilution. Virus A was inactivated at 59° C. in two trials and at 60° in two trials, while virus B was inactivated at 74° in one trial, at 76° in two trials, and at 78° in one trial. Thus virus B was distinctly the more tolerant to heat. Virus A was inactivated in vitro at 20° at 7 days in one trial and at 8 days in two trials, while virus B was inactivated at 4 days in one trial, at 5 days in one trial, and at 6 days in two trials. Thus virus A was definitely more resistant to aging than virus B.

If the properties of cabbage virus A are compared with those of the cabbage mosaic virus described by Larson and Walker [9] on the basis of reaction on tobacco, some degree of resemblance is found. The cabbage mosaic virus preparation, which, it is now presumed, was a mixture of viruses A and B, was inactivated by dilution to 1–2,000, by heating at 55° C. for 10 minutes, and by aging at 20° to 22° for 72 hours. In each case it was somewhat less tolerant to the treatment than was cabbage virus A. When an extract taken from plants infected with both A and B viruses was used on tobacco, as shown in table 1, the results were very similar to those obtained with virus A alone. Whether the differences are very significant is questionable. Judging from the number of local lesions per leaf caused by the untreated extracts, the concentration of virus A in the cabbage mosaic preparation used by Larson and Walker [9] was much less than that in the extracts used for the present cabbage virus A determinations. This may account for some of the discrepancy between the two determinations.

In the tests of Tompkins, Gardner, and Thomas,[10] cabbage black ring virus was inactivated by heat at 59° C., by dilution at 1–1,000, and in vitro at 3 days. When a culture obtained from Tompkins was run concurrently with the tests reported here, as shown in table 1, inactivation by heat was at 59°, by dilution at 1–15,000, and by aging at 7 days. Allowing for discrepancies due to differences in handling and methods, it is obvious that cabbage virus A and the black ring virus were very similar in the physical properties studied.

Of the crucifer viruses previously described, cauliflower mosaic virus [11] conformed most closely in properties, symptoms, and host range to cabbage virus B. The former was reported by Tompkins as inactivated by dilution at 1–3,000, by heat at 75° C., and in vitro after aging 15 days at 22°. When a culture of cauliflower mosaic virus obtained from Tompkins was tested concurrently with virus B, it was found it was inactivated by dilution at 1–2,000, by heat at 78°, and by aging at 20° at 11 days (table 2). Except for reaction to aging, the two viruses were very close to each other in the physical properties studied.

[9] See footnote 3.
[10] See footnote 5.
[11] See footnote 4.

SYMPTOMS AND HOST RANGE

It is obvious that the symptoms on various hosts described for cabbage mosaic by Larson and Walker[12] were concerned with the reaction of the two viruses, cabbage viruses A and B, when cruciferous hosts were involved. When noncruciferous hosts were used, only cabbage virus A was concerned, since in this investigation cabbage virus B was not found to infect any but cruciferous plants. The assumption that the cabbage virus previously described [12] is a mixture of cabbage viruses A and B is based on the fact that in practically every case in which cabbage mosaic has been collected during midseason in southeastern Wisconsin and in the Puget Sound cabbage-seed-growing area of western Washington both viruses have been found to be present. In the cool weather of late autumn in Wisconsin virus A declines in amount. In view of the circumstances just cited, it was considered advisable to restudy the effect of the two viruses separately and together on the same list of hosts previously recorded. Furthermore, in view of the possible relation of viruses A and B both to the black ring virus and to the cauliflower mosaic virus, these too were included in the studies.

It became evident early in the work that cabbage virus A was favored by relatively high air temperatures, while cabbage virus B was favored by relatively low air temperatures. Therefore the host-range studies were conducted simultaneously in a greenhouse controlled at 28° C. and in one at 20°, and each species was inoculated simultaneously with all viruses.

HOST RANGE OF THE VIRUSES

The symptoms on cruciferous hosts in response to each virus and to viruses A and B in combination are given in table 3. It is to be noted that all four viruses were infectious to all cruciferous species tested except yellow rocket, hoary alyssum, dames violet, and Virginia stock. The last three mentioned were infected by cabbage virus A and the black ring virus but not by cabbage virus B and the cauliflower mosaic virus, and the first was not infected by any of them.

The cauliflower mosaic virus and cabbage virus B infected no noncruciferous plants, while cabbage virus A and the black ring virus infected many such hosts belonging to numerous genera and families (table 4). When viruses A and B in combination were inoculated to susceptible noncruciferous hosts, the symptoms were identical with those produced when virus A alone was used.

Over this wide range of cruciferous and noncruciferous hosts both virus A and the black ring virus were found to be infectious on all species susceptible to either and tested with both, with the exception of *Solanum integrifolium*, which was not infected by the black ring virus. Neither virus infected by the mechanical method Jerusalem-cherry, bittersweet, jimsonweed, belladonna, cherry pepper, hyacinth-bean, scarlet runner bean, soybean, cowpea, morning-glory, okra, four-o'clock, knapweed, French marigold, yellow rocket, or sweet alyssum.

Thus it is apparent that cabbage virus A and black ring virus are very similar in their host range. Likewise virus B and the cauliflower mosaic virus are similar in host range and are both confined to hosts within the Cruciferae.

[12] See footnote 3.

TABLE 3.—*Symptoms produced on young plants of various species of Cruciferae in the greenhouse at constant temperatures of 20° and 28° C. when inoculated mechanically with cabbage virus A, cabbage black ring virus, cabbage virus B, cauliflower mosaic virus, and cabbage viruses A and B together*

Scientific and common names	Symptoms resulting from inoculation with—				
	Cabbage virus A	Black ring virus	Cabbage virus B	Cauliflower mosaic virus	Cabbage viruses A and B
Brassica oleracea var. *capitata* L. (cabbage, var. Jersey Queen).	Local: None. Systemic: At 28° C. mild chlorotic mottle, with or without vein clearing, rapidly becoming severe and diffuse until mostly chlorotic with small dark-green islands; leaf-bloom reduction moderate; petioles somewhat elongated; severe leaf malformation; premature leaf abscission; no necrosis; severe stunting. At 20° young leaves symptomless or with occasional chlorotic spots; chlorotic lesions more distinct than at 28°; mild mottle; mild necrosis; slight stunting.	Local: None. Systemic: Similar to virus A but milder. At 28° C. mottle finer and less severe; vein clearing more common, stunting, distortion, and leaf-bloom reduction less than for virus A; round, dark-green spots with chlorotic halos or dark-green rings bounded on either side by light-green zones; no necrosis. At 20° rings and ring spots more conspicuous than with virus A; moderate to severe necrosis as black spots and black rings; mottle milder than at 28°.	Local: None. Systemic: At 28° C. mild vein clearing increasing until prominent, then receding until barely discernible; mild diffuse mottle; leaf-bloom reduction moderate, decreasing with age; no necrosis; no distortion; symptoms becoming masked in time; slight stunting. At 20° vein clearing slower but similar, becoming prominent sometimes as chlorotic vein banding; mild mottle; no stunting; enations sporadic; symptoms less pronounced with age but never completely masked as at 28°.	Local: None. Systemic: Similar to virus B but always milder.	Local: None. Systemic: At 28°C. at first same as virus A; vein clearing becoming prominent and mottle more severe but of much finer type than with virus A, leaves taking on a yellow-white speckled appearance; severe necrosis as streaking of veins and blue-black sunken flecks in parenchyma; severe stunting. At 20° mottle milder, vein clearing more prominent and more persistent than at 28°; chlorotic vein banding common; necrosis, stunting, leaf-bloom reduction milder than at 28° but more severe than with virus A at 20°.
B. oleracea var. *botrytis* L. (cauliflower, var. Early Snowball).	Local: None at 28°. Sporadic irregular necrotic pattern at 20°. Systemic: At 28° coarse chlorotic mottle, occasional vein clearing; midrib curvature; unilateral leaf development on some plants; very mild purplish rings at margins of chlorotic spots on lower side of leaf, causing various patterns; mild stunting. At 20° mild vein clearing; mild mottle; very slight stunting; purple rings much more prominent than at 28°.	Local: None at 28°. At 20° prominent chlorotic rings and ring spots common, becoming necrotic with age. Systemic: At 28° similar to virus A, but mottle milder, chlorotic lesions more distinct, less distortion, slightly more stunting. At 20° ring necrosis much more severe than at 28° and than virus A at 20°; on young leaves numerous but small whitish rings developing on upper surface.	Local: None. Systemic: At 28° vein clearing decreasing, diffuse mottle increasing with age; characteristic midrib curvature; mild stunting. At 20° pronounced vein clearing and vein banding; mild diffuse mottle; midrib curvature; very mild stunting; no necrosis.	Local: None. Systemic: Symptoms similar to virus B but decidedly more pronounced.	Local: None. Systemic: At 28° initia symptoms identical with those of virus A but vein clearing of virus B becoming more prominent and mottle more severe; leaf malformation and stunting more severe than with virus A. At 20° similar to 28° with milder mottle and stunting but more pronounced vein clearing and necrotic rings.

TABLE 3.—*Symptoms produced on young plants of various species of Cruciferae in the greenhouse at constant temperatures of 20° and 28° C. when inoculated mechanically with cabbage virus A, cabbage black ring virus, cabbage virus B, cauliflower mosaic virus, and cabbage viruses A and B together*—Continued

Scientific and common names	Symptoms resulting from inoculation with—				
	Cabbage virus A	Black ring virus	Cabbage virus B	Cauliflower mosaic virus	Cabbage viruses A and B
B. oleracea var. *botrytis* L. (broccoli, var. Italian Green Sprouting).	Local: None. Systemic: At 28° similar to cabbage except slight stunting and leaf malformation; no premature defoliation. At 20° mottle less diffuse; vein clearing more prominent; stunting very mild.	Local: None at 28°. At 20° small chlorotic spots later forming an irregular necrotic pattern. Systemic: At 28° coarse chlorotic mottle much as in cabbage; no necrosis. At 20° mottle milder but necrosis developing with age; distinctly more necrotic than with virus A.	Local: None. Systemic: At 28° similar to cabbage with mild stunting. At 20° vein clearing and fine mottle; stunting less than at 28°.	Local: None. Systemic: Similar to but milder than virus B at first, gradually becoming indistinguishable from virus B.	Local: None. Systemic: At 28° similar to cabbage. No necrotic spots or patterns as described by Larson and Walker,[1] possibly because plants were discarded too soon.
B. oleracea var. *gongylodes* L. (kohlrabi, var. Early White Vienna).	Local: None. Systemic: At 28° similar to cabbage except less malformation and stunting and formation of necrotic rings or spots conspicuous on older leaves. At 20° chlorotic mottle; some streak along veins; premature leaf abscission. No evidence of reduction in size of storage organ.	Local: None at 28°. At 20° irregular necrotic pattern on inoculated leaves. Systemic: At 28° numerous chlorotic rings, later giving rise to a coarse mottle as in cabbage; no necrosis; symptoms milder than on cabbage. At 20° numerous small chlorotic rings giving a marble mosaic effect; necrotic ringing increasing with age; very similar to virus A.	Local: None. Systemic: At 28° same type as for cabbage but very mild. At 20° similar to cabbage, more severe than at 28° but still very mild, slightly more pronounced than those of cauliflower mosaic virus.	Local: None. Systemic: Of same type as virus B but slightly less conspicuous.	Local: None. Systemic: At 28° similar to cabbage; mottle more diffuse and chlorosis more pronounced than for virus A; scattered necrotic spots; symptoms generally quite mild. At 20° fairly mild diffuse mottle; conspicuous vein clearing.
B. oleracea var. *viridis* L. (kale, var. Dwarf Green Curled).	Local: Necrosis on inoculated leaves of some plants. Systemic: At 28° similar to cabbage with irregular chlorotic blotches quite extensive; stunting very slight. At 20° similar to 28° except chlorotic blotches more distinct.	Local: None at 28°. At 20° necrosis as an irregular pattern. Systemic: At 28° mild but coarse chlorotic mottle; no necrosis; slight stunting. At 20° mottle is very mild; necrosis becoming conspicuous with age; no chlorotic blotches as for virus A.	Local: None. Systemic: At 28° similar to cabbage. At 20° similar to cabbage except fleck necrosis on some plants.	Local: None. Systemic: Similar to virus B.	Local: None. Systemic: At 28° similar to cabbage except streak on veins common; symptoms relatively mild; stunting mild. At 20° similar to 28° except necrosis quite severe.

[1] See footnote 3.

B. oleracea var. *gemmifera* DC. (brussels sprouts, var. Long Island Mammoth).	Local: None at 28°. At 20° scattered chlorotic lesions. Systemic: At 28° similar to but milder and slower than on cabbage; streak necrosis. At 20° similar to cabbage except necrotic rings similar to cauliflower developing after 3 to 4 weeks.	Local: None at 28°. At 20° severe necrosis causing entire leaf to die. Systemic: At 28° indistinguishable from virus A. At 20° few chlorotic lesions, rapidly giving way to a severe systemic necrosis; markedly different from virus A at 20°.	Local: None. Systemic: Similar to cabbage, very mild at 28°; vein clearing prominent at 20°.	Local: None. Systemic: Very similar to those of virus B.	Local: None. Systemic: At 28° similar to but more severe than virus A. At 20° vein clearing and vein banding more conspicuous than at 28°.
B. rapa L. (turnip, var. Purple Top White Globe).	Local: None. Systemic: At 28° vein clearing; diffuse mottle becoming severe; marked leaf malformation; severe stunting; root and hypocotyl enlargement almost completely inhibited; highly lethal, killing majority of plants before appearance of symptoms. At 20° symptoms milder throughout.	Not tested.	Local: None. Systemic: At 28° slower than virus A; temporary vein clearing; severe mottle and chlorosis; unilateral leaf distortion; severe leaf stunting and savoying; root and hypocotyl enlargement suppressed; lethal. At 20° mild vein clearing for extended period followed later by severe mottle; symptoms more severe than at 28°; lethal.	Local: None. Systemic: Similar to those for virus B except more severe early; later virus B symptoms reached identical stage.	Local: None. Systemic: At 28° most plants killed outright; others showed more diffuse mottle than virus A and more pronounced vein clearing. At 20° more prominent vein clearing, more diffuse mottle, and more severe stunting than virus A.
B. campestris L. (wild yellow mustard).	Local: At 28° local lesions and severe necrosis on old plants. Systemic: At 28° vein clearing; chlorotic mottle; severe stunting; highly lethal; severe necrosis on old plants. At 20° vein clearing and mottle; severe stunting; highly lethal.	Local: As for virus A. Systemic: As for virus A except less necrosis at 28°. Severe stunting, chlorosis, vein clearing and leaf distortion; death occurs later than for virus A. At 20° very similar to virus A except slightly more severe.	Local: None. Systemic: At 28° prominent vein clearing, followed tardily by midrib curvature and necrotic flecking; moderate stunting. At 20° vein clearing and general chlorosis; stunting more severe than at 28°. Not lethal at 28° or 20°.	Local: None. Systemic: Similar to virus B but more rapid and severe; highly lethal.	Local: None. Systemic: At 28° vein clearing and diffuse mottle, former increasing in prominence; unilateral leaf malformation; severe stunting; highly lethal. At 20° similar to 28° but milder.
B. hirta Moench (*B. alba* (L.) Rabenh. (white mustard).	Local: None. Systemic: At 28° young leaves rolled as if burnt; mottle of green and whitish areas; rapid necrosis spreading along veins; highly lethal. At 20° similar to 28°.	Local: None. Systemic: Severe chlorotic mottle and vein clearing but no necrosis; stunting and leaf distortion very severe. Plants not dead in 3 weeks after inoculation as for virus A. At 20° symptoms slightly more severe than at 28°.	Local: None. Systemic: At 28° vein clearing; chlorotic spots; stunted, curled leaves; tan necrotic spots; severe stunting; killing of growing point; highly lethal. At 20° mild stunting and distortion, vein clearing, rosetting; highly lethal.	Local: None. Systemic: Similar to virus B but more pronounced, more stunting; marked rosetting; very highly lethal.	Local: None. Systemic: At 28° similar to virus A; at 20° similar to virus A but more severe.

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TABLE 3.—*Symptoms produced on young plants of various species of Cruciferae in the greenhouse at constant temperatures of 20° and 28° C. when inoculated mechanically with cabbage virus A, cabbage black ring virus, cabbage virus B, cauliflower mosaic virus, and cabbage viruses A and B together*—Continued

Scientific and common names	Symptoms resulting from inoculation with—				
	Cabbage virus A	Black ring virus	Cabbage virus B	Cauliflower mosaic virus	Cabbage viruses A and B
B. nigra (L.) Koch (black mustard).	Local: Necrotic spots on inoculated leaves of old plants. Systemic: At 28° vein clearing and chlorosis with rapid necrosis, rosetting, and death. At 20° similar but slightly slower.	Local: Angular necrotic lesions on inoculated leaves; more conspicuous at 20° than 28°. Systemic: At 28° plants becoming chlorotic and dying as if burnt by fire; death in 5 to 6 days after inoculation. At 20° plants stunted, leaves curled inward, growing point becoming necrotic and causing plant to die; much more severe than virus A.	Local: None. Systemic: At 28° vein clearing and mild mottle; severe rosetting; leaf curvature; chlorosis; stunting. At 20° similar to 28° but milder.	Local: None. Systemic: Similar to virus B but much more rapid and severe.	Local: None on young plants. Systemic: Similar to virus A.
Capsella bursa-pastoris (L.) Medic. (shepherds-purse).	Local: None. Systemic: At 28° vein clearing and diffuse mottle; lower leaves yellow and necrotic; leaves malformed; florets blighted; severe stunting; commonly lethal. At 20° similar to 28° and equally severe.	Not tested.	Local: None. Systemic: At 28° vein clearing conspicuous; coarse yellow mottle; mild stunting; general chlorosis.	Local: None. Systemic: Very similar to virus B.	Local: None. Systemic: Similar to virus A but becoming more severe with age.
Matthiola incana var. *annua* (L.) Voss (annual stock, var. Dwarf Large Flowering Ten Weeks).	Local: None. Systemic: At 28° vein clearing and diffuse mottle; leaf malformation; necrosis along and between veins; flowers breaking; stunting. At 20° similar to 28°, but less severe.	Local: None at 28°. At 20° conspicuous necrotic lesions 1 to 2 mm. in diameter, later forming necrotic ring spots. Systemic: At 28° vein clearing and mottling very similar to virus A; considerable leaf distortion. At 20° vein clearing, distortion and mottling more severe than at 28°; also more severe than virus A at 20°; vein necrosis and leaf distortion marked.	Local: None. Systemic: At 28° vein clearing, mild mottle, occasional chlorotic spots, slight stunting. At 20° vein clearing and chlorotic spots more pronounced; very mild stunting; no flower breaking.	Local: None. Systemic: Similar to virus B but slightly more pronounced.	Local: None. Systemic: At 28° similar to virus A but more severe stunting, malformation, necrosis, and defoliation. At 20° milder than at 28° but more severe than virus A at 20°.

Raphanus sativus L. (radish, vars. Scarlet Globe, Crimson Giant, and French Breakfast).	Local: None Systemic: At 28° vein clearing, disappearing rapidly; mild mottle; moderate stunting; hypocotyl enlargement retarded; some tan necrotic flecks. At 20° vein clearing with fine mottle; moderate stunting.	Local: None Systemic: Similar to virus A but milder with only a mild indistinct mottle.	Local: None Systemic: At 28° conspicuous vein clearing, fine mottle, mild stunting and malformation. At 20° vein clearing and mild mottle, both slightly more prominent than at 28°.	Local: None Systemic: Similar to virus B but rapidly becoming more pronounced and severe than virus B; some necrosis.	Local: None. Systemic: At 28° similar to virus A with vein clearing becoming more prominent as with virus B. At 20° same except for bright red necrotic flecks with yellow halos.
Lepidium sativum L. (cress, var. Extra Curled).	Local: None Systemic: At 28° rapid severe stunting and yellowing followed by necrosis and death. At 20° similar to virus A but slightly slower.	Local: None Systemic: Exactly as for virus A.	Local: None Systemic: At 28° and 20° vein clearing and diffuse mottle; moderate stunting.	Local: None Systemic: Indistinguishable from virus B.	Local: None. Systemic: Same as for virus A.
L. virginicum L. (wild peppergrass).	Local: None Systemic: At 28° vein clearing and mottle; small necrotic spots; entire plant turning purple, wilting, and dying. At 20° similar but milder.	Local: Circular necrotic lesions, more conspicuous at 20° than 28°. Systemic: At 28° chlorotic vein clearing followed by distorting mottle; plants severely stunted and chlorotic; less severe than virus A. At 20° similar to 28° but vein clearing and mottling more conspicuous; more severe than at 28°.	Local: None Systemic: At 28° and 20° vein clearing becoming prominent; diffuse mottle; golden chlorotic lesions on old leaves.	Local: None Systemic: Indistinguishable from virus B.	Local: None. Systemic: Same as for virus A.
Thlaspi arrense L. (pennycress).	Local: Necrotic lesions on inoculated leaves at 28°. Systemic: At 28° vein clearing and general chlorosis; marginal necrosis of young leaves; brown discoloration of veins and veinlets; lethal. At 20° similar but slightly slower.	Local: As for virus A Systemic: At 28° and 20° chlorotic vein clearing, severe golden-yellow chlorosis; severe stunting followed by death; distinctly more severe than virus A. Slightly milder at 20° than at 28°.	Local: None Systemic: At 28° vein clearing; fine mottle turning to golden yellow; lethal. At 20° similar but more pronounced vein clearing and early necrosis; partly lethal.	Local: None Systemic: Indistinguishable from virus B.	Exactly similar to virus A.
Neslia paniculata (L.) Desv. (ballmustard).	Local: None Systemic: At 28° and 20° vein clearing and general chlorosis, some lower leaves rapidly turning yellow and abscising, others becoming necrotic; highly lethal.	Local: None at 28°; at 20° tan necrotic lesions. Systemic: At 28° plants show conspicuous chlorotic vein clearing, yellow mottle, and leaf curvature; death within 10 days after inoculation; more severe than for virus A. At 20° similar to 28° but less severe.	Local: None Systemic: At 28° and 20° vein clearing, followed by mild mottle.	Local: None Systemic: Similar to virus B.	Local: None. Systemic: Indistinguishable from virus A.

TABLE 3.—*Symptoms produced on young plants of various species of Cruciferae in the greenhouse at constant temperatures of 20° and 28° C. when inoculated mechanically with cabbage virus A, cabbage black ring virus, cabbage virus B, cauliflower mosaic virus, and cabbage viruses A and B together*—Continued

Scientific and common names	Symptoms resulting from inoculation with—				
	Cabbage virus A	Black ring virus	Cabbage virus B	Cauliflower mosaic virus	Cabbage viruses A and B
Sisymbrium altissimum L. (tumblemustard).	Local: None Systemic: At 28° mild mottle, stunting, and unilateral development of leaves; necrosis on lower leaves; symptoms quite mild. At 20° plants stunted and rosetted; leaves mottled; old leaves necrotic.	Not tested	Local: None Systemic: At 28° and 20° mild yellow mottle and vein clearing; midrib curvature in young leaves.	Local: None Systemic: Very similar to virus B.	Local: None. Systemic: Similar to virus A.
Brassica juncea (L.) Cosson (Indian mustard).	Local: None Systemic: At 28° vein clearing; mild mottle becoming chlorotic and necrotic; highly lethal. At 20° similar to 28°; highly lethal.	Local: As described for black mustard. Systemic: As described for black mustard.	Local: None Systemic: At 28° vein clearing followed by mild mottle, narrowing and wrinkling of leaves. At 20° similar to 28° but finally more severe.	Local: None Systemic: Similar to virus B but much more severe.	Local: None. Systemic: Similar to virus A.
B. kaber var. *pinnatifidum* (Stokes) L. C. Wheeler (charlock).	Local: None at 28°. At 20° a few angular necrotic lesions. Systemic: At 28° leaves curling and turning dark green; rapid necrosis and death. At 20° similar to 28° but slightly slower.	Local: As described for black mustard. Systemic: As described for black mustard.	Local: None Systemic: Vein clearing; chlorotic streaks along veins; midrib curved; leaves distorted. At 20° vein clearing and mild mottle, becoming more severe with age.	Local: None Systemic: Similar to virus B but more rapid and more severe, developing into severe chlorosis and rosette.	Local: None. Systemic: Similar to virus A.
B. napus L. (rape, var. Dwarf Essex).	Local: At 28° occasional zonate brown necrotic spots on inoculated leaves, necrosis spreading along veins, petiole, and stem; becoming lethal. Systemic: At 28° vein clearing and severe mottle and malformation, including reduction of leaf lamina to "shoestring" and formation of blisterlike pockets; severe chlorosis. At 20° similar to 28° but less severe.	Local: None Systemic: At 28° similar to virus A but less distortion and diffuse chlorosis; no necrosis. At 20° mild fine-type mottle; much milder than for 28°; necrosis on lower leaves more severe than with virus A; lower leaves distinctly chlorotic.	Local: None Systemic: At 28° vein clearing; prominent, diffuse mottle; midrib curved. At 20° incubation period longer, symptoms more pronounced; stunting mild.	Local: None Systemic: Similar to virus B but more rapid in development and markedly more severe. At 28° considerable chlorosis and leaf ruffling. At 20° marked rosetting of young growth.	Local: Same as virus A. Systemic: At 28° similar to virus A for about 3 weeks when vein clearing similar to virus B develops; severe stunting. At 20° similar to 28° but slightly less severe.

B. pekinensis (Lour.) Rupr. (Chinese cabbage, var. Chihli).	Local: None Systemic: At 28° persistent vein clearing; rapidly developing chlorotic mottle; severe malformation and stunting; tan necrotic spots coalescing or purplish streak of veins extending to petiole and stem; often lethal. At 20° severe diffuse mottle; stunting and distortion.	Local: None Systemic: Very similar to virus A except stunting, necrosis, and leaf malformation more severe.	Local: Occasional chlorotic lesions produced on inoculated leaves, more so at 20° than at 28°. Systemic: Marked vein clearing; conspicuous chlorotic lesions; necrosis along margins of young leaves; marked curvature of young leaves; rosetting. At 20° similar to 28° but milder.	Local: Same as for virus B. Systemic: Similar to virus B but much more severe. Rosetting more severe at 20° than at 28°.	Local: None. Systemic: At 28° similar to virus A; tan necrosis more severe, mottle more diffuse. At 20° vein clearing more persistent than with virus A.
Berteroa incana (L.) DC. (hoary alyssum).	Local: None Systemic: At 28° mottle consisting mostly of yellow with green along veins and veinlets; vein clearing; leaf malformation; florets blighted. At 20° diffuse mottle, malformation; slight stunting.	Local: None Systemic: Mottle and vein clearing very similar to virus A at 28° and 20°.	None	None	Local: None. Systemic: Similar to virus A.
Barbarea vulgaris R. Br. (yellow rocket).	None	None	do	do	None.
Hesperis matronalis L. (dames violet).	Local: None Systemic: At 28° very conspicuous yellow mottle and vein clearing; leaves severely distorted and stunted; slight vein necrosis. At 20° symptoms similar to 28° but milder.	Local: None Systemic: Similar to virus A but mottle less conspicuous, less distortion and stunting.	do	do	Local: None. Systemic: Same as for virus A.
Iberis amara L. (candytuft).	Local: None Systemic: Mild vein clearing; mottling; downward curling of young leaves; stunting.	Local: None Systemic: Indistinguishable from virus A.	Not tested	Not tested	Not tested.
Alyssum maritimum Lam. (sweet alyssum).	None	None	do	do	Do.
Malcomia maritima (L.) R. Br. (Virginian stock).	Local: Circular, tan, necrotic lesions 1 to mm. in diameter. Systemic: Mild mottle; chlorosis; new growth markedly rosetted due to stunting and stimulated growth of adventitious buds; not lethal at 20°.	Local: As for virus A but more pronounced. Systemic: Similar to virus A but decidedly more severe; lethal at 20°.	None	None	Do.

TABLE 3.—*Symptoms produced on young plants of various species of Cruciferae in the greenhouse at constant temperatures of 20° and 28° C. when inoculated mechanically with cabbage virus A, cabbage black ring virus, cabbage virus B, cauliflower mosaic virus, and cabbage viruses A and B together*—Continued

Scientific and common names	Symptoms resulting from inoculation with—				
	Cabbage virus A	Black ring virus	Cabbage virus B	Cauliflower mosaic virus	Cabbage viruses A and B
Matthiola bicornis (Sibth. and Sm.) DC. (evening scented stock).	Local: Numerous circular chlorotic lesions 1 to 2 mm. in diameter. Systemic: Vein clearing, mottling, leaf curvature and stunting; plants bleached, becoming necrotic and dying 20 to 25 days after inoculation at 20°.	Local: Conspicuous necrotic lesions 2 to 3 mm. in diameter. Systemic: As for virus A but more severe; plants died 12 days after inoculation at 20°.	Not tested	Not tested	Do.
Cheiranthus cheiri L. (wallflower).	Local: None Systemic: Chlorotic mottle accompanied by severe leaf and stem curvature; young severely stunted leaves curved unilaterally and downward, forming a marked rosette; more pronounced at 28° than at 20°.	Local: None Systemic: As for virus A except slightly more severe.	do	do	Do.

TABLE 4.—*Symptoms produced on young noncruciferous plants in the greenhouse at constant temperatures of 20° or 28° C. when inoculated mechanically with cabbage virus A and the cabbage black ring virus*

Scientific and common names	Symptoms resulting from inoculation with—	
	Cabbage virus A	Black ring virus
Nicotiana tabacum L. (tobacco, var. Connecticut Havana No. 38).	Local: Necrotic lesions appearing as small flecks but rapidly expanding to 5 to 8 mm. in diameter; brick-red centers and brown, concentric outlying rings; proximal lesions coalescing to form large necrotic area but still maintaining their individuality; appearing in 3 to 4 days at 28° C. and in 7 to 8 days at 20°. Systemic: None	Local: As for virus A. Systemic: None.
N. glutinosa L.	Local: At 28° conspicuous pattern of chlorotic rings and ring spots; no necrosis. At 20° large chlorotic lesions becoming necrotic. Systemic: At 28° zonate chlorotic rings and ring spots; no mottle; no leaf distortion. At 20° numerous chlorotic spots and rings producing a distinct mottle developing into a severe systemic necrosis as the chlorotic lesions become necrotic; considerable stunting and distortion of young leaves.	Local: As for virus A except more rapid in development. Systemic: As for virus A except more severe stunting and less severe necrosis at 20° C.
N. multivalvis Pursh.	Local: None Systemic: Chlorotic mottle with considerable distortion of leaf laminae; mild stunting; no necrosis at 28° or 20°.	Local: At 20° small whitish rings about pencil point in size. Systemic: Conspicuous pattern of white rings or pattern of irregular etchings in addition to mottle characteristic of virus A; large, yellow, ring spot lesions on older leaves; stunting and necrosis severe, many plants killed; much more severe than virus A; more severe at 20° than at 28°.
N. paniculata L.	Local: At 28° lesions ranging from tan spots to tan rings 1 to 2 mm. in diameter, expanding and developing into pattern of large concentric rings and ring spots; no necrosis. Systemic: None	Local: As for virus A except for tendency of concentric rings to develop necrotic centers. Systemic: None.
N. quadrivalvis Pursh.	Local: None Systemic: At 28° conspicuous mottle with considerable stunting and distortion of leaves; no necrosis; no etching pattern as with black ring virus.	Local: None. Systemic: Mottle as for virus A; white etching pattern as in *N. multivalvis* but less pronounced.
N. rustica L.	Local: None at 28°. Inconspicuous chlorotic lesions, occasionally necrotic at 20°. Systemic: At 28° chlorotic rings and solid, irregular chlorotic spots; some rings zonate; with age ring development ceasing and only chlorotic spots developing; some leaf distortion. At 20° only irregular chlorotic lesions developing, giving a coarse mottle effect.	Local: Few chlorotic lesions at 28°. Conspicuous chlorotic rings and necrotic lesions at 20°. Systemic: Much like virus A except zonate rings more prevalent than in virus A. At 20° numerous chlorotic rings and ring spots tending to become necrotic; decidedly more conspicuous than at the higher temperature.
N. acuminata (Graham) Hook.	Local: At 28° tan chlorotic lesions tending to become necrotic. Systemic: Chlorotic lesions, later becoming necrotic; young leaves severely puckered and drawn.	Local: At 28° tan necrotic lesions with a dark-brown periphery and surrounded by a chlorotic halo. Systemic: As for virus A except more necrotic.
N. alata var. *grandiflora* Comes.	Local: At 28° whitish rings and ring spots tending to become necrotic. Systemic: None	Local: At 28° necrotic lesions surrounded by white rings. Systemic: None

TABLE 4.—*Symptoms produced on young noncruciferous plants in the greenhouse at constant temperatures of 20° or 28° C. when inoculated mechanically with cabbage virus A and the cabbage black ring virus*—Continued

Scientific and common names	Symptoms resulting from inoculation with—	
	Cabbage virus A	Black ring virus
× *N. sanderae* W. Wats.	Local: At 20° circular tan chlorotic lesions tending to consist of rings; developing large, diffuse, chlorotic areas with age; no necrosis. Systemic: None.	Local: At 20° as for virus A except for tendency of lesions to develop necrotic borders. Systemic: None.
N. sylvestris Speg.	Local: At 28° necrotic lesions very similar to those on *N. tabacum*, but developing 8 to 10 days later. Systemic: None.	Local: At 28° as for virus A but slightly more rapid in development. Systemic: None.
N. trigonophylla Dunal.	Local: At 20° numerous chlorotic rings and lesions. Systemic: Large chlorotic lesions tending to consist of 6 to 7 concentric chlorotic rings; no necrosis; no distortion.	Local: At 20° as for virus A. Systemic: Similar to virus A but lesions largely spots with bright-yellow centers and little or no ringing.
N. longiflora Cav.	Local: At 28° whitish rings 1 to 2 mm. in diameter. Large golden-yellow spots on old plants. Systemic: None.	Local: At 28° as for virus A except rings 2 to 4 mm. in diameter; large yellow-green spots on old plants. Systemic: None.
N. nudicaulis S. Wats.	Local: At 28° none. Systemic: Very mild mottle with lesions, tending to become necrotic; symptoms very mild.	Local: At 28° none. Systemic: Only slight chlorosis along the veins; virus recovered in high concentration.
Physalis pubescens L. (husk tomato).	Local: At 28° circular chlorotic lesions. Systemic: Numerous chlorotic lesions and vein clearing resulting in a true mottle effect; young leaves severely stunted and distorted; severe defoliation, leaving only a tuft of stunted leaves at the growing point.	Local: As for virus A. Systemic: Exactly as for virus A except for conspicuous chlorotic ringing and vein banding on some plants.
Solanum integrifolium Poir. (Chinese scarlet eggplant).	Local: Black, irregular to circular, necrotic lesions and streaking of the veins at both 28° and 20°. Systemic: At 28° and 20° much as on inoculated leaves; no mottle; no vein clearing; only systemic necrotic lesions and vein streaking with some puckering of leaf midrib; severe stunting.	Local: None. Systemic: No infection in 4 trials.
S. rostratum Dunal (buffalo-bur).	Local: At 28° few faintly necrotic lesions remaining inconspicuous. Systemic: None.	Local: Conspicuous small, brown, necrotic lesions at 28°. Systemic: None.
S. pseudo-capsicum L. (Jerusalem-cherry).	None.	None.
S. dulcamara L. (bittersweet).	do.	Do.
Datura metel L.	Local: At 28° broad, irregular chlorotic areas along and between the veins; interveinal chlorotic lesions. Systemic: Numerous circular, chlorotic lesions on the leaves with slight puckering of the leaf laminae.	Local: As for virus A. Systemic: Exactly as for virus A.
D. meteloides Dunal.	Local: At 28° circular chlorotic lesions. Systemic: Conspicuous mottle with mildly stunted leaves.	Local: As for virus A. Systemic: Exactly as for virus A.
D. stramonium L. (jimsonweed).	None.	None.
Atropa belladonna L. (belladonna).	do.	Do.
Capsicum frutescens var. *cerasiforme* (Mill.) Bailey (cherry pepper).	do.	Do.

TABLE 4.—*Symptoms produced on young noncruciferous plants in the greenhouse at constant temperatures of 20° or 28° C. when inoculated mechanically with cabbage virus A and the cabbage black ring virus*—Continued

Scientific and common names	Symptoms resulting from inoculation with—	
	Cabbage virus A	Black ring virus
Lycium halimifolium Mill. (matrimony-vine).	Local: At 28° tan chlorotic leisons never becoming necrotic. Systemic: None.	Local: Tan necrotic lesions. Systemic: None.
Nicandra physalodes (L.) Pers. (apple-of-Peru).	Local: At 28° few small, chocolate-brown lesions increasing in size, becoming irregular in outline and finally surrounded by chlorotic halos. Systemic: None.	Local: As for virus A except much more numerous and developing more rapidly. Systemic: None.
Salpiglossis sinuata Ruiz and Pav.	Local: None. Systemic: At 28° very mild indistinct mottle; conspicuous vein clearing; mild stunting; no necrosis.	Local: None. Systemic: Mild but distinct fine-type mottle; no conspicuous vein clearing; moderate stunting; no necrosis.
Beta vulgaris var. *cicla* L. (Swiss chard, var. Lucullus).	Local: Tan to red chlorotic flecks, occasionally necrotic; slightly more pronounced at 28° than at 20°. Systemic: At 28° brick-red to tan spots occasionally surrounded by yellow halos; slight stunting; at 20° similar to 28°.	Local: Severe brick-red necrotic flecking; distinctly more necrotic than virus A. Systemic: As for virus A but necrosis more severe than virus A at both 28° and 20°.
B. vulgaris L. (sugar beet, var. U. S. 200 × 215).	Local: At 28° tan to red chlorotic to necrotic flecks. Systemic: Small, circular or irregular, brown to red necrotic lesions up to 2 mm. in diameter, sometimes coalescing; occasionally a faint mottle.	Local: Red necrotic flecking more severe than virus A. Systemic: As for virus A but more severe.
Spinacia oleracea L. (spinach, vars. Old Dominion, Giant Thick Leaf, Bloomsdale).	Local: None. Systemic: At 28° vein clearing, chlorotic mottle; young leaves rapidly becoming chlorotic; stunted, puckered; growing points becoming a rosette of "shoestring" leaves; severe stunting; lower leaves becoming necrotic and abscising; stunted rosette dying in 3 to 4 weeks. At 20° slower, diffuse mottle, and vein clearing; moderate stunting and leaf distortion.	Not tested.
Amaranthus tricolor L. (pigweed).	Local: Small but conspicuous necrotic lesions at 28° and 20°. Systemic: Conspicuous, chlorotic vein clearing and diffuse mottle; stunting and premature leaf abscission.	Local: As for virus A. Systemic: As for virus A except no mottle.
Celosia cristata L. (cockscomb).	Local: At 20° tan necrotic lesions 2–4 mm. in diameter. Systemic: Slight stunting and leaf distortion; very mild mottle.	Local: As for virus A except appearing 4–5 days earlier. Systemic: Exactly as for virus A.
Chenopodium album L. (lambsquarters).	Local: At 28° tan, circular necrotic lesions 1–2 mm. in diameter, tan centers with dark-brown borders somewhat zonate. Systemic: Small chlorotic lesions becoming necrotic; stunting; distortion.	Local: As for virus A. Systemic: As for virus A but slightly more severe.
Dolichos lablab L. (hyacinth-bean)	None	None.
Phaseolus coccineus L. (scarlet runner bean).	do	Do.
Soja max (L.) Piper (soybean)	do	Do.
Vigna sinensis (Torner) Hassk (cowpea, var. Blackeye).	do	Do.

TABLE 4.—*Symptoms produced on young noncruciferous plants in the greenhouse at constant temperatures of 20° or 28° C. when inoculated mechanically with cabbage virus A and the cabbage black ring virus*—Continued

Scientific and common names	Symptoms resulting from inoculation with—	
	Cabbage virus A	Black ring virus
Ipomoea purpurea (L.) Roth. (morning-glory).	None do	None. Do.
Digitalis purpurea L. (foxglove)	Local: None Systemic: At 28° inconspicuous chlorosis along the veins; few scattered chlorotic lesions; symptoms very mild.	Local: None. Systemic: Chlorosis along veins; mottle very conspicuous in some plants, much more so than virus A.
Hibiscus esculentus L. (okra, var. Long Velvet).	None	Not tested.
Abutilon theophrasti Medic. (velvet leaf).	Local: None Systemic: At 20° mild chlorotic mottle becoming masked with age; slight stunting but no necrosis or leaf malformation.	Local: None. Systemic: As for virus A except mottle less conspicuous; virus not removed in inoculation to tobacco.
Mirabilis jalapa L. (four-o'clock)	None	None.
Verbena hybrida Voss (garden verbena).	Local: At 20° necrotic lesions 5 to 6 mm. in diameter, with tan centers and chocolate-brown peripheries. Systemic: Mild mottle, chlorosis and slight leaf distortion.	Local: At 20° as for virus A except lesions more numerous. Systemic: Mild mottle, chlorosis, leaf distortion, and vein necrosis.
Centaurea nigra L. (knapweed)	None	None.
Tagetes patula L. (French marigold).	do	Do.
Vinca minor L. (periwinkle)	Local: None Systemic: At 28° circular chlorotic lesions with tan borders producing a ring effect; no stunting or distortion.	Local: None. Systemic: As for virus A except rings more numerous and more conspicuous.
Stellaria media (L.) Cyrill. (chickweed.	Local: None Systemic: At 20° mild mottle and chlorotic vein banding; no stunting; no necrosis.	Local: None. Systemic: As for virus A except mottle more conspicuous and leaves slightly distorted.
Scabiosa atropurpurea L. (mourning bride).	Local: None Systemic: At 20° chlorotic mottle with vein clearing; leaves stunted and distorted; no necrosis.	Local: None. Systemic: As for virus A except much more severe; mottle and necrosis severe.
Godetia amoena Lilja (farewell-to-spring).	Local: None Systemic: At 28° conspicuous chlorotic mottle; stunting and distortion of young leaves; proliferation of adventitious growth in some plants.	Local: None. Systemic: Very similar to virus A.
Delphinium ajacis L. (rocket larkspur).	Local: At 28° crescent-shaped necrotic lesions along leaf margin. Systemic: Chlorosis, yellow mottle, necrosis as on inoculated leaves; necrosis of stem and floral buds; plants severely stunted and with a scorched appearance; flower breaking in form of white streaks in the corolla.	Local: As for virus A but developing 7 to 10 days earlier. Systemic: As for virus A but 7 to 10 days earlier; more pronounced than virus A in early stages but ultimately quite similar.
Reseda odorata L. (mignonette)	Local: At 28° chlorotic lesions occasionally necrotic. Systemic: Conspicuous vein clearing and mild mottle; marked stunting and distortion of young leaves; severe necrosis of veins causing entire leaves to die on some plants.	Local: Necrotic lesions, only occasionally chlorotic. Systemic: As for virus A except necrosis more severe.
Calendula officinalis L. (calendula, var. Orange King).	Local: None Systemic: At 20° chlorotic mottle; stunting, distortion, and general chlorosis of young leaves; necrotic spotting on some leaves.	Local: None. Systemic: Indistinguishable from virus A.

TABLE 4.—*Symptoms produced on young noncruciferous plants in the greenhouse at constant temperatures of 20° or 28° C. when inoculated mechanically with cabbage virus A and the cabbage black ring virus*—Continued

Scientific and common names	Symptoms resulting from inoculations with—	
	Cabbage virus A	Black ring virus
Centaurea moschata L. (sweet-sultan).	Local: None. Systemic: At 20° mottle with vein clearing; severe stunting and distortion; not lethal.	Local: None. Systemic: As for virus A except development much more rapid and severe; lethal at 20° within 3 weeks after inoculation.
Dimorphotheca aurantiaca DC. (winter Cape-marigold).	Local: None. Systemic: At 28° chlorotic vein clearing and mottle; marked distortion of young leaves; rapidly lethal.	Local: None. Systemic: Exactly as for virus A.
Helianthus annuus L. (sunflower, var. Giant Russian Mammoth).	Local: Black necrotic lesions 3 to 4 mm. in diameter with tan centers and black peripheries at both 28° and 20°. Systemic: Numerous chlorotic lesions becoming necrotic; necrosis finally involving entire leaf causing it to abscise; sypmtoms alike at 20° and 28°.	Local: As for virus A. Systemic: Exactly as for virus A.
Senecio cruentus DC. (florist's cineraria).	Local: Tan necrotic rings at 28° and 20°. Systemic: Mild vein clearing and chlorosis of young leaves; stunting and distortion very mild; milder at 20° than at 28°.	Local: Irregular necrotic pattern but not rings as in virus A. Systemic: Conspicuous vein clearing; mild mottle; vein necrosis; considerable distortion. More severe at 28° and 20° than virus A.

SYMPTOMS ON CRUCIFEROUS HOSTS

The symptoms of the various viruses on the different cruciferous hosts are given in table 3. It may be well to point out here some of

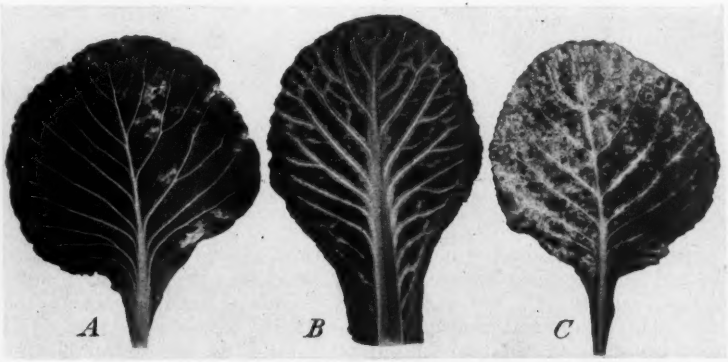

FIGURE 1.—Leaves from cabbage plants systemically infected: *A*, Cabbage virus A at 28° C.; *B*, cabbage virus B at 16°; *C*, cabbage viruses A and B at 28°. Photographed 5 weeks after inoculation. Note the mottle effect of virus A at 28° and the intensification of mottle at this temperature when virus B is present. Note also the vein clearing caused by virus B at 16°.

the cardinal points of similarity and contrast. Cabbage virus B had a longer incubation period by several days than cabbage virus A. The latter usually first caused a mottle at 28° C. (fig. 1, *B*) and,

although vein clearing was often evident, it tended to be obscured rather promptly as the mottle became more pronounced. Necrosis was not common with virus A on cabbage but appeared as purple rings on cauliflower and streak necrosis on brussels sprouts; leaf malformation was common. On wild mustards, pennycress, and peppergrass the disease caused by virus A progressed rapidly and was usually lethal. At 20° this virus was generally distinctly milder than the black ring virus in rate of progress and severity of symptoms, although necrosis and premature defoliation were sometimes more pronounced on certain hosts than at 28°.

Cabbage virus B usually caused vein clearing and a mild diffuse mottle at 28° C. Vein clearing increased in prominence, but

FIGURE 2.—Enations on cabbage leaves systemically infected with cabbage virus B. Photographed 60 days after inoculation and incubation at 12° C.

eventually all symptoms other than stunting and chlorosis became masked at this temperature. At 20° vein clearing appeared more slowly than at 28°, but it gradually increased until it became the prominent permanent characteristic. At 16° vein clearing was still more pronounced than at 20° (fig. 1, *B*). At low temperatures enations on systemically infected leaves of cabbage were common (fig. 2). It may not be amiss, therefore, to refer to virus B as the vein clearing virus and to virus A as the mottle virus.

When viruses A and B were inoculated together the symptoms were very similar to those described earlier for cabbage mosaic,[13] and they were more truly representative of the disease as it occurred in nature

[13] See footnote 3.

in southeastern Wisconsin. When plants were kept at 28° C. the symptoms were most like those of virus A. As a rule, however, mottle was more severe and necrosis more common and destructive (fig. 1, *C*). It is evident that at the high temperature virus A predominated, but the symptoms were more severe than when only virus A was present. At 20° the course of the disease was retarded as compared with 28° and the symptoms of virus B gradually became predominant. In such plants as white mustard, black mustard, and charlock, in which the action of virus A was rapid and lethal at 28° and nearly as severe at 20°, there was little or no evidence of any influence of the slowly acting virus B on the course of the disease.

The symptoms produced by cabbage virus B and the cauliflower mosaic virus were in general very similar, giving further evidence

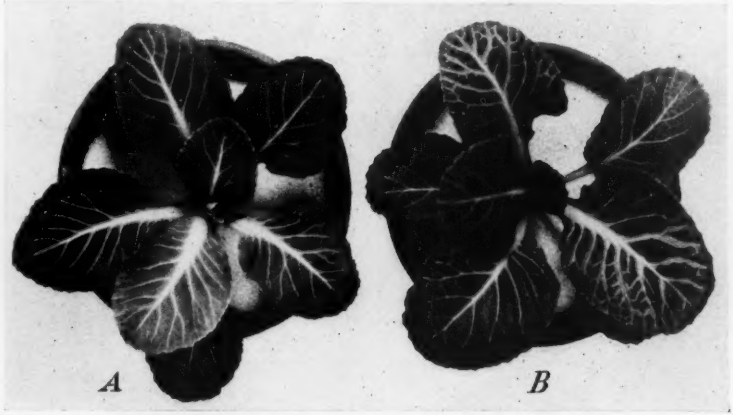

FIGURE 3.—Cabbage plants showing effect of the cauliflower mosaic virus (*A*) and of the cabbage virus B (*B*), 42 days after inoculation at 12° C. Note the more prominent vein clearing with cabbage virus B.

that they are closely related. Certain differences, however, are worthy of note. On cabbage the cauliflower mosaic virus was distinctly milder in its effect (fig. 3), while on cauliflower the reverse was true. On broccoli the cauliflower mosaic virus was definitely milder at first but gradually became indistinguishable from virus B. On turnip the cauliflower mosaic virus was the more severe early, but gradually the two became indistinguishable. On kale, brussels sprouts, cress, peppergrass, pennycress, ballmustard, and tumble-mustard the two were nearly identical. On yellow mustard, white mustard, black mustard, Indian mustard, charlock, rape, Chinese cabbage, and radish the cauliflower mosaic virus caused more rapid disease development and the symptoms were much more severe (fig. 4).

The black ring virus produced symptoms rather similar to those of cabbage virus A. There were some marked differences, however, which are worthy of note. On cabbage the black ring virus was less pronounced at 28° C. but more severe at 20°, while with cabbage virus A the reverse was true. At the latter temperature black spots

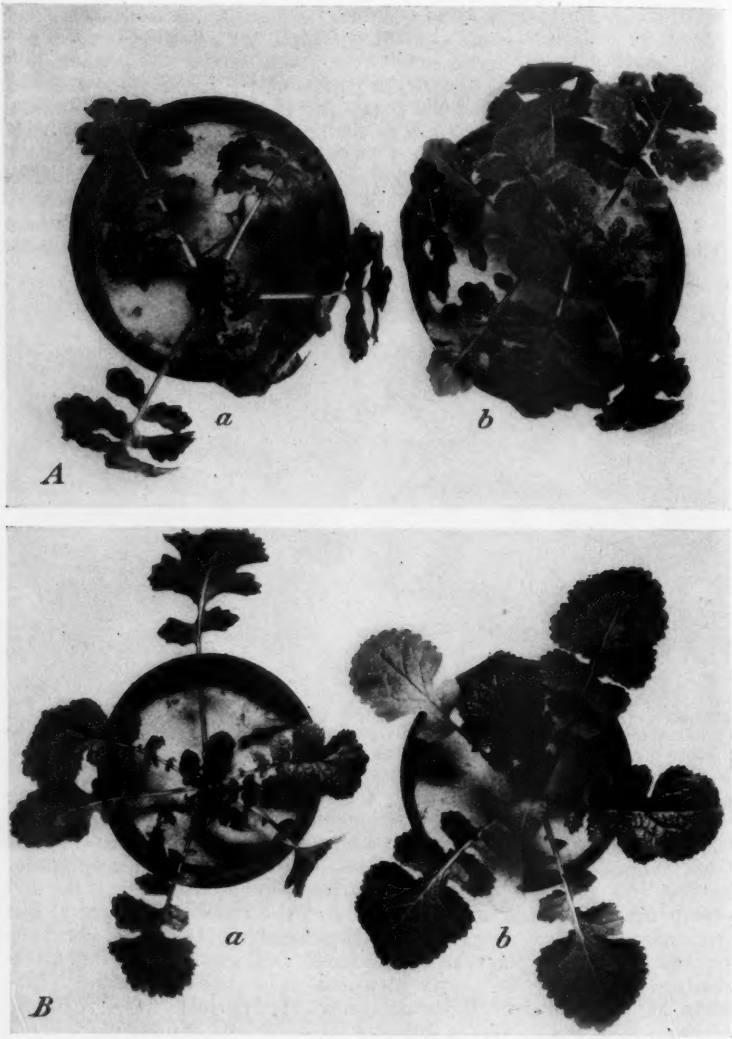

FIGURE 4.—Wild mustards showing effects of the cauliflower mosaic virus (*a*) and of the cabbage virus B (*b*), 21 days after inoculation. *A*, White mustard plants at 28° C. Note extreme rosetting caused by the cauliflower virus. *B*, Indian mustard plants at 20°. The symptoms of virus B on this host become more pronounced after longer incubation but never reach the severity of those associated with the cauliflower virus.

and black rings were characteristic of the black ring virus, while chlorotic spots with relatively mild necrosis were the more common with virus A. This relative difference also prevailed on cauliflower.

SYMPTOMS ON NONCRUCIFEROUS HOSTS

As already pointed out, cabbage virus B and cauliflower mosaic virus caused no symptoms on noncruciferous plants. Cabbage virus A and black ring virus had identical host ranges as far as tested, except that the latter did not affect Chinese scarlet eggplant. The black ring virus had an incubation period shorter by 1 to 7 days on some hosts. Symptoms of the two viruses (table 4) could usually be distinguished but in most cases only in minor respects. The progression of symptoms was usually more rapid with black ring virus in that necrosis appeared first and the disease reached its most severe stage first. On some hosts the appearance of chlorotic rings and ring spots and the transition of the rings into necrotic areas were pronounced with the black ring virus and relatively inconspicuous with virus A. As a rule, under the conditions of the greenhouse tests, black ring infection resulted in more necrosis and the death of more plants than did infection with virus A.

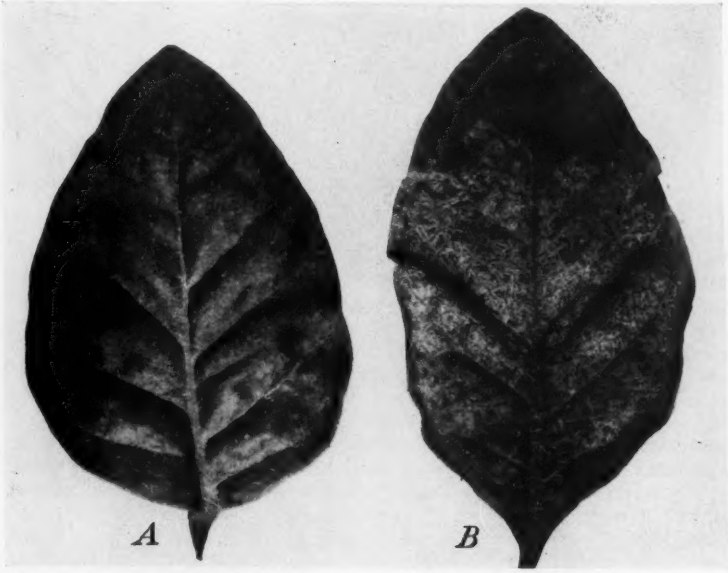

FIGURE 5.—Systemically infected leaves of *Nicotiana multivalvis* showing effects of cabbage virus A (*A*) and of the black ring virus (*B*). Note the very mild chlorotic mottle in *A* caused by cabbage virus A and the marked etching pattern in *B* caused by the black ring virus.

Certain more distinctive symptoms were to be noted in some hosts. In *Nicotiana multivalvis*, for instance, a conspicuous pattern of white rings was produced by the black ring virus in addition to the type

of chlorotic mottle produced by virus A (fig. 5). A similar distinction was noted with *N. quadrivalvis*. On the other hand, in the case

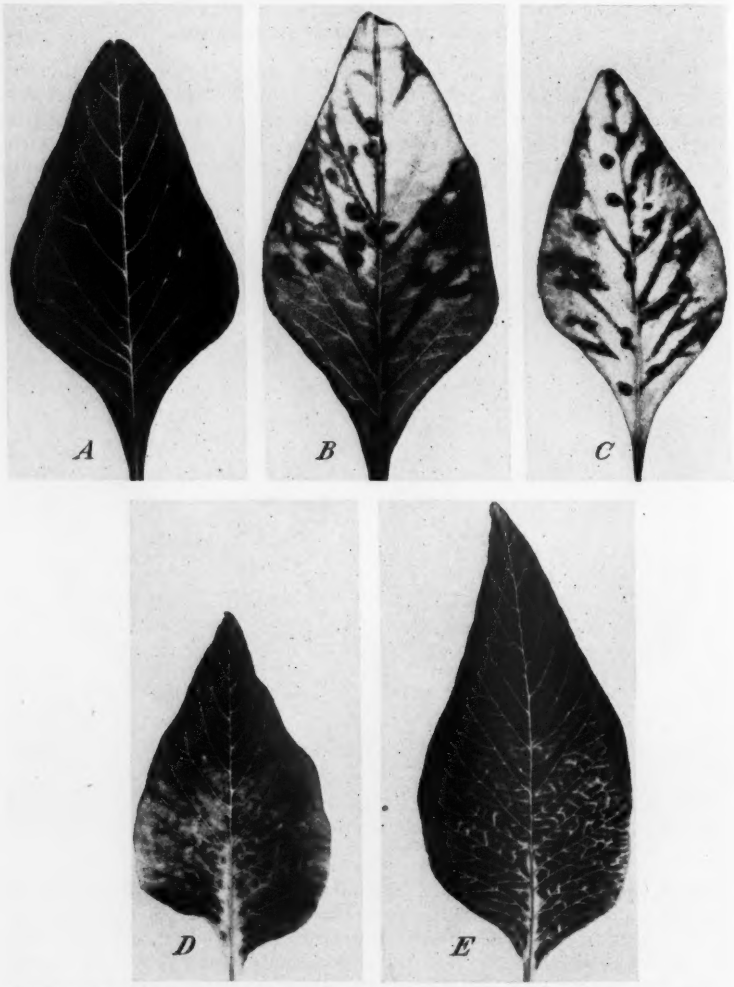

FIGURE 6.— Leaves of *Amaranthus tricolor* showing the effects of cabbage virus A and of the black ring virus. *A*, Uninoculated leaf. *B*, Leaf inoculated with cabbage virus A. *C*, Leaf inoculated with the black ring virus. Note pigmented lesions on a diffuse chlorotic background. *D*, *E*, Systemically infected leaves from the plants that produced leaves shown in *B* and *C*, respectively. Note diffuse mottle with virus A and chlorotic vein clearing with the black ring virus.

of *Amaranthus tricolor* conspicuous pigmented lesions appeared on inoculated leaves with both viruses, while on systemically infected

leaves there was a diffuse mottle with virus A and a chlorotic vein clearing with the black ring virus (fig. 6).

As is to be expected, the inoculation of each noncruciferous host with a mixture of cabbage viruses A and B led to the same results as inoculation with virus A alone. This is in contrast to the results obtained with cruciferous hosts susceptible to both viruses in which a combined pathological effect was often distinct from and more severe than that of each virus when inoculated separately.

DISCUSSION

At the present time there is much confusion in the differentiation and classification of plant viruses. Some of this is due to the inadequate methods of defining them. While symptoms, host ranges, and such physical properties as those indicated by inactivation of virus extracts by aging in vitro, by heat, and by dilution are helpful, they may lead to overemphasis of relatively minor differences. The constant danger that more than one virus may be involved in a disease complex adds to the confusion when differentiation by these methods alone is involved.

The results reported here show that two distinct viruses are concerned in cabbage mosaic as found in the Middle West and in western Washington. One of the components is shown to resemble the black ring virus described in California as to host range, properties, and symptoms, while the other closely resembles the cauliflower mosaic virus described in that State. It is equally obvious from a study of the disease reaction of many hosts that in the case of each pair the viruses are not identical. The distinctions between the black ring virus and cabbage virus A on the one hand and between the cauliflower mosaic virus and cabbage virus B on the other are concerned primarily with disease progression and incubation period rather than with physical properties and host range. Thus, the cauliflower mosaic virus causes more severe disease reaction in cauliflower than in cabbage, while with cabbage virus B the reverse is true. Many other points of distinction are to be found. Cabbage virus A causes typically chlorotic and mottle effects, while the black ring virus causes dark-green rather than chlorotic rings and necrotic ring spots.

It seems only logical to conclude, therefore, that cabbage virus A and the black ring virus are closely related strains. They are, undoubtedly, also closely related to the virus described by Hoggan and Johnson [14] from turnip. According to the Johnston system of classification, it is the belief of the writers that they should all be classed under turnip virus 1 Hoggan and Johnson. The ring necrosis virus of Larson and Walker [15] has a very similar host range, although the properties reported are different. It is believed, therefore, that this is also another strain of the same virus.

The cauliflower mosaic virus is obviously quite distinct from turnip virus 1. Cabbage virus B is undoubtedly a strain of the cauliflower virus. The virus from broccoli described from southern England recently by Caldwell and Prentice [16] appears to be closely

[14] HOGGAN, I. A., and JOHNSON, J. A VIRUS OF CRUCIFERS AND OTHER HOSTS. Phytopathology 25: 640-644, illus. 1935.

[15] See footnote 6.

[16] CALDWELL, J., and PRENTICE, I. W. A MOSAIC DISEASE OF BROCCOLI. Ann. Appl. Biol. 29: 366-373, illus. 1942.

related. This group of strains is referred to as cauliflower virus 1, and various other strains of it will undoubtedly be found.

It is obvious that when the cabbage A strain of turnip virus 1 and the cabbage B strain of cauliflower virus 1 infect cabbage together the disease differs in many aspects from that which results when either affects this host alone. The combined effect is that which occurs naturally in the Middle West in the disease known as cabbage mosaic.

SUMMARY

Cabbage mosaic as it occurs in the Middle West has been shown to consist of two causal virus entities tentatively designated as cabbage viruses A and B. These two viruses were also recovered from diseased plants in western Washington.

Studies of symptoms, host ranges, and physical properties show that cabbage virus A is very similar to but nevertheless consistently distinct from the black ring virus described from California,[17] while cabbage virus B is very similar to but distinct in some respects from the cauliflower mosaic virus also described from California.[18]

It is suggested that cabbage virus A, the cabbage black ring virus, and the cabbage ring necrosis virus are all strains of turnip virus 1 Hoggan and Johnson. It is further suggested that the cauliflower mosaic virus as described by Tompkins[18] be designated as cauliflower virus 1 and that cabbage virus B and the broccoli virus of Caldwell and Prentice[19] be designated as strains thereof.

[17] See footnote 5.
[18] See footnote 4.
[19] See footnote 16.

RESPONSE OF SHORTLEAF AND PITCH PINES TO SOIL AMENDMENTS AND FERTILIZERS IN NEWLY ESTABLISHED NURSERIES IN THE CENTRAL STATES [1]

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INTRODUCTION

In the early years of production of pine planting stock in the Central States much difficulty was experienced in nurseries newly established on land previously used for agriculture. In many cases only a few small clumps of seedlings survived in each seedbed and height growth of the seedlings was very irregular. The needles on some seedlings were yellow, on others purple, instead of the normal green. Loss from damping-off was excessive before emergence and for a week or two afterward. A thin crust of alkaline soil formed on some surfaces as a result of concentration of calcium salts from capillary and sprinkling water. Most of the soils were low in organic matter. Some were alkaline because of lime natural to the site or distributed during grading operations. Some drainage difficulties were encountered. For these reasons experiments in soil amendment and fertilization were undertaken in three Forest Service nurseries located at Chillicothe, Ohio, Vallonia, Ind., and Licking, Mo. All the nursery areas had previously been in farm crops; consequently there was no information regarding their soil deficiencies for pine seedlings. Cross-sectional research therefore seemed more suitable than isolated studies on specific problems. This paper covers the results of the first 5 years' experimentation.

DESCRIPTION OF NURSERIES

The Chillicothe nursery is situated on a terrace of the preglacial Tuyes River, the topography of which was changed from level to rolling by terminal moranic deposits of the Illinoian glacier. The material of the rolling surface is predominantly sandy in texture, but not homogeneous. Exposure in a cut nearby shows a 12- to 18-inch sandy loam surface horizon with a tight subsoil of sand, gravel, and clay, and sand and gravel below this to a depth of approximately 20 feet. The nursery area itself includes a hill of sandy material without much subsoil development, highly calcareous throughout its profile. Below the zone of moranic influence lies terrace material of a heavier texture. The surface soil is yellowish brown in color, the subsoil predominantly reddish brown but lighter in some spots. In its undisturbed state, this soil probably should be classified as Holston sandy loam.

As the topography was a little too rolling for easy tillage, the surface was leveled. The topsoil was scraped and piled, then replaced after the leveling operation. Unfortunately, more colloidal material was present than might have been expected in a soil of such light

[1] Received for publication September 23, 1943.

FIGURE 1.—Two views of seedlings in the same seedbed and nearby vegetation in the Chillicothe nursery 1937: *A*, good pitch pine seedlings and poor clover, on acid soil (pH 5); *B*, poor pitch pine seedlings and luxuriant clover, on sweet soil (pH 8)

surface texture, and on many areas scraping exposed the subsoil. Replacing the surface material did not, of course, restore the original normal drainage and aeration. The natural structure had been destroyed by compaction with heavy grading machinery. Grading had exposed calcium carbonate and spread it over a portion of the area. On this portion the surface soil had a reaction above neutrality

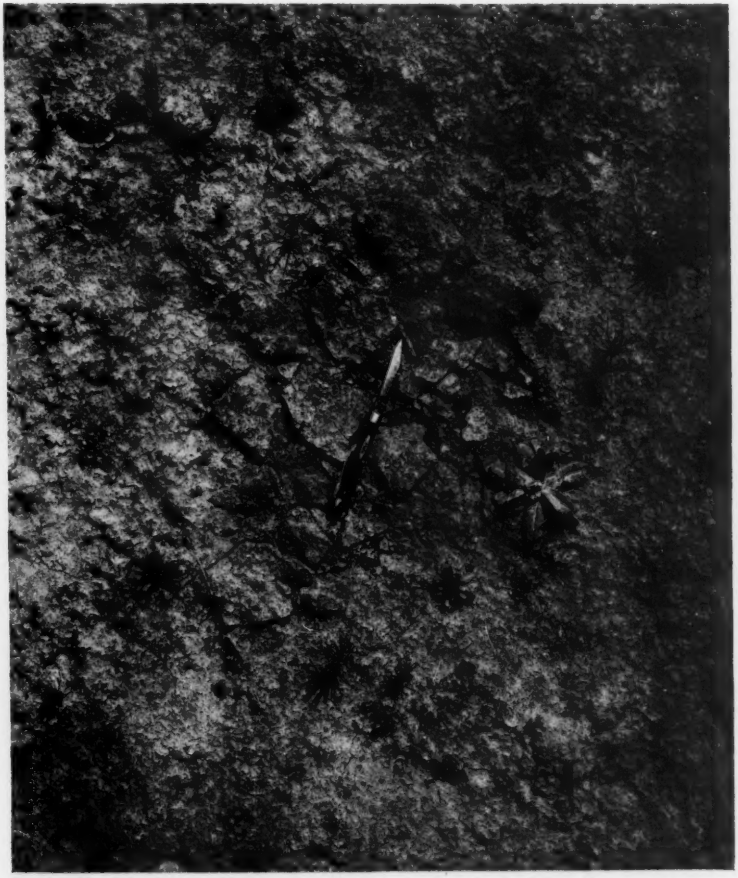

FIGURE 2.—Crusting of soil as a result of concentration of calcareous salts in soil water. Clillicothe nursery, 1937

which affected pine seedlings adversely (fig. 1). In general, however, excessive alkalinity was confined to a surface crust not more than a quarter of an inch thick (fig. 2) and the subsurface soil had a reaction of pH 5 to 6. The surface crusting had taken place as a result of concentration of calcium salts from evaporation of soil water.

The Vallonia nursery is located in Jackson County, Ind., on a portion of one of the Shelbyville moraines of the early Wisconsin

glaciation Its site is composed of high terraces, gently sloping except for a dunelike hill of wind-blown sand. The soil has not been surveyed officially, but would probably be classified as Princeton sandy loam. The surface material is a yellowish brown sand to sandy loam, becoming more yellow with depth. At 10 feet some clay streaks occur and the texture is heavier. All parts of the area have good drainage. The soil is deficient in organic matter and in the inorganic colloidal fraction; hence base-exchange capacity is limited. At Vallonia, as at Chillicothe, the surface soil was alkaline, and after rain or watering a shallow crust frequently developed from concentration of soluble salts. A considerable part of the incrusting material had been formed by evaporation of sprinkling water, which came from calcareous material.

The nursery at Licking, Mo., lies chiefly on a terrace of Huntington silt loam, one corner extending onto a gentle slope of Hanceville fine sandy loam. The soil is somewhat heavier than the soils of the Chillicothe and Vallonia nurseries. Consequently it has an advantage over them in capacity for retaining soluble fertilizers, owing to higher colloid content. Here the sprinkling water was not excessively calcareous, the soil was slightly acid, and surface crusting did not take place.

EXPERIMENTAL PLAN AND PROCEDURE

The exploratory experiments reported here were designed to answer questions regarding (1) nutrient deficiencies in the soil limiting thrift of pine seedlings; (2) quantities of the various elements needed; (3) form of fertilizing material best suited to nursery use; (4) time of application most favorable; (5) a method of adjusting soil acidity; (6) effect of incoporating peat into the soil; (7) influence of soil reaction on fertilizer effects; and (8) effects of inoculation with pine duff.

The plan of experimentation was practically the same in all cases. Nursery beds 4 feet wide were marked off into as many 3-foot sections as were necessary for the projected number of tests. If the number of plots was large the bed was first divided into blocks. By allocating each replicate to a separate block, any possible influence of variation in soil from one end of the nursery bed to the other was reduced. Fertilizers were applied in dry form, by working them into the surface soil before seeding, or in solution after seeding. Seedling density counts and height measurements on each plot were taken on a 1-foot strip crossing the plot at its midpoint. At digging time this 1- by 4-foot strip of trees was undermined to prevent breaking the roots. The seedlings were allowed to fall into the trench and were then washed and bundled, and the bundles were labeled with plot numbers. In the laboratory, the roots and tops of the seedlings were measured and weighed.

Averages for single plots were compared with the general average for all plots in the bed. To avoid confusing increase in height from crowding with that directly attributable to fertilizer, a preliminary test for covariance between height and density was made, and if it appeared, a correction was introduced.

1936 EXPERIMENT

At the Vallonia nursery the sprinkling water was modified in 1936 by adding sulfuric acid. This reduced the soil reaction temporarily, but was discontinued because of the danger of excessive leaching of

bases. Another expedient tried was to work acid-saturated peat into the soil. A core of soil 4 inches deep was mixed with a core of peat of the same diameter 1 inch deep. Sufficient water was added to the soil-peat mixture, in a large beaker, to allow easy stirring, and 1/10 normal sulfuric acid was introduced from a burette. When the reaction, tested by use of a glass electrode, reached pH 5.8 the quantity of acid used was recorded. From this and the weight of peat, the quantities of acid and peat necessary for application in practice were calculated. This treatment remedied the crusting and alkalinity. Peat also acted as a buffer against accumulation of calcium carbonate. This addition, together with less frequent but heavier applications of sprinkling water, solved the calcium problem.

1937 EXPERIMENTS

CHILLICOTHE NURSERY

The first of the series of fertilizer experiments was started at Chillicothe in the spring of 1937 with pitch pine (*Pinus rigida*). Potassium sulfate, ammonium nitrate, and phosphoric acid were applied in solution in amounts equivalent to 100 pounds per acre of potassium, nitrogen, and phosphorus, respectively. Seven treatments, N, P, K, NP, NK, KP, and NPK, and no treatment were assigned at random to eight plots in each of eight blocks in one long nursery bed. The entire lay-out was then quartered and peat applied at the rate of about 0.2 pound per square foot to two of the quarters, randomly selected. Each of the four quarters was then halved and 2 gallons of a turbid solution of calcium hydroxide applied to each of four of the eight halves, randomly selected.

Counts were made in July, and counts and height measurements were made in September. Both sets of data are presented in table 1. No significant effect of peat on height growth or density of seedlings appeared in this experiment, hence averages according to application of peat are not included in table 1.

TABLE 1.—*Average density in July and September 1937, of pitch pine seedlings in Chillicothe nursery plots, seeded in the spring of 1937*

Fertilizer [1]	Average seedling density per square foot					Average seedling height [3] in September 1937
	July 1937			September 1937		
	Limed [2] plots	Unlimed plots	All plots	Limed [2] plots	Unlimed plots	
	Number	*Number*	*Number*	*Number*	*Number*	*Inches*
Nitrogen, phosphorus, and potassium	12.6	10.0	11.3	8.4	8.7	1.9
Nitrogen and phosphorus	13.5	9.2	11.3	7.8	8.1	1.9
Nitrogen and potassium	27.0	8.3	17.6	18.4	6.6	1.3
Potassium and phosphorus	28.1	31.2	29.6	28.8	32.8	1.9
Nitrogen	20.1	11.8	16.0	11.1	8.8	1.3
Phosphorus	26.3	25.4	25.8	22.2	23.4	1.7
Potassium	19.7	30.0	24.8	27.4	29.4	1.3
None	21.1	24.8	23.0	19.7	23.5	1.3
Minimum mean difference significant at—						
5-percent level	8.8		12.4	7.05		.27
1-percent level	11.9		16.8	9.57		.37

[1] Applied at rate of 100 pounds of each element per acre.
[2] Turbid solution of calcium hydroxide applied at rate of 2 gallons to 12 square feet.
[3] Height measurements were made in thirtieths of a foot, converted to inches, and rounded off.

The outstanding observation in this experiment is the effect of nitrogen. For nitrogen and every fertilizer treatment including nitrogen, seedling density of unlimed plots averaged less than for no treatment. Neither potassium nor phosphorus alone influenced density on unlimed plots significantly as measured in July, but association of increased density with potassium was indicated by the September counts on both limed and unlimed plots. Phosphorus and potassium together caused the highest density recorded in the experiment. Where nitrogen was added to phosphorus or to the potassium-phosphorus combination, density was reduced. Peat did not appreciably increase density, neither did it reduce it. The same is true of lime alone, but the interaction of lime and some fertilizer treatments did influence density. Lime reduced the injurious effect of nitrogen. Immediately after addition of lime to soil treated with ammonium nitrate the odor of ammonia was noticed, indicating liberation of some ammonia and consequent reduction of total quantity of nitrogen.

When seedlings were measured in September, it was not observed that nitrogenous fertilizers in conjunction with soil amendments had had any injurious effects on height growth.

The nitrogen-phosphorus combination gave superior height, although it gave the lowest fall density on limed plots (table 1). Potassium alone did not increase height significantly, but the combination of potassium and phosphorus and that of nitrogen, potassium, and phosphorus did. Apparently the increase in height growth was associated with phosphorus. This soil had an abundance of red oxide of iron, but either rigid fixation of phosphorus by iron did not occur or the soil was sufficiently alkaline to render any iron phosphate so formed relatively soluble and thus available to pine seedlings.

VALLONIA NURSERY

At the Vallonia nursery an experimental seeding of shortleaf pine (*Pinus echinata*) was made in the fall of 1937 to test response to (1) some of the minor elements, (2) potassium and phosphorus at normal levels of application, (3) an excessive quantity of an organic nitrogenous fertilizer, (4) three levels of phosphorus, (5) phosphorus at three levels of soil reaction, and (6) peat incorporated in the surface soil. The lay-out consisted of 2 blocks of 39 plots each. One block received peat at the rate of about 0.2 pound per square foot (4 tons per acre), the other none. Each of these large blocks was divided into 3 smaller blocks of 13 plots each, to which the following treatments were assigned at random: Copper sulfate, manganese sulfate, magensium sulfate, zinc sulfate, and boric acid each at the rate of 60 pounds of the element per acre; potassium sulfate at the rate of 120 pounds of potassium per acre; soybean meal at the rate of 400 pounds of nitrogen per acre; treble superphosphate at the rate of 100 pounds of phosphorus per acre for each of three levels of reaction (pH 4, 6, and 8); treble superphosphate at the rate of 40 and 500 pounds of phosphorus per acre in soil at pH 6; and no treatment.

Introduction of a more efficient sprinkling system at the Vallonia nursery in 1937 made unnecessary any further treatment to prevent concentration of lime salts in the surface soil.

The seedlings were counted in May of 1938 and were counted and measured for height early in September of that year; the results are presented in table 2. In the spring of 1939 they were lifted, washed, measured, and weighed. The results are presented in table 3.

TABLE 2.—*Average measurements in May 1938 and in the fall of 1938, of shortleaf pine seedlings in Vallonia nursery plots, seeded in the fall of 1937*

Fertilizer and rate of application per acre	Soil reaction	Average seedling density per square foot in May 1938 on plots having—		Average seedling measurements in fall of 1938 on plots having—			
				Peat		No peat	
		Peat	No peat	Density per square foot	Height	Density per square foot	Height
	pH	*Number*	*Number*	*Number*	*Inches*	*Number*	*Inches*
1. Copper sulfate, to give 60 pounds Cu	6	71	35	10.8	4.8	4.2	3.3
2. Manganese sulfate, to give 60 pounds Mn	6	73	34	12.9	5.5	4.8	2.8
3. Magnesium sulfate, to give 60 pounds Mg	6	69	35	8.8	4.8	6.1	3.0
4. Zinc sulfate, to give 60 pounds Zn	6	41	29	12.8	5.8	3.4	2.7
5. Potassium sulfate, to give 120 pounds K	6	43	40	11.3	6.5	6.4	3.2
6. Boric acid, to give 60 pounds B	6	44	52	9.4	4.8	6.7	3.7
7. Soybean meal, to give 400 pounds N	6	7	4	1.7	4.9	1.0	.8
8. Treble superphosphate, to give 100 pounds P	4	50	25	9.1	7.7	4.5	7.4
9. Treble superphosphate, to give 100 pounds P	8	37	16	5.7	5.2	2.1	3.4
10. Treble superphosphate, to give 40 pounds P	6	49	34	7.2	6.7	4.5	5.8
11. Treble superphosphate, to give 100 pounds P	6	62	26	13.5	7.4	4.4	7.2
12. Treble superphosphate, to give 500 pounds P	6	64	32	13.8	7.9	4.1	9.4
13. None	6	83	28	10.3	4.9	2.3	2.4
Minimum mean difference significant at—							
5-percent level		44	27	5.0	1.62	3.8	1.46
1-percent level		61	37	8.5	2.19	5.3	2.06

TABLE 3.—*Average measurements in the spring of 1938, of shortleaf pine seedlings in Vallonia nursery plots, seeded in the fall of 1937*

Fertilizer and rate of application per acre	Soil reaction	Average seedling measurements in spring of 1939 on plots having—											
		Peat						No peat					
		Length		Green weight		Dry weight		Length		Green weight		Dry weight	
		Root	*Top*	*Root*	*Top*	*Root*	*Top*	*Root*	*Top*	*Root*	*Top*	*Root*	*Top*
1. Copper sulfate, to give 60 pounds Cu	*pH* 6	*In.* 13.7	*In.* 8.6	*Gm.* 6.0	*Gm.* 10.5	*Gm.* 2.11	*Gm.* 3.07	*In.* 10.4	*In.* 5.5	*Gm.* 3.3	*Gm.* 5.6	*Gm.* 0.97	*Gm.* 1.38
2. Manganese sulfate, to give 60 pounds Mn	6	12.9	8.2	5.5	9.8	1.74	2.59	8.9	3.8	1.6	2.4	.56	.66
3. Magnesium sulfate, to give 60 pounds Mg	6	12.9	8.3	5.5	10.3	1.72	2.64	12.0	6.8	6.2	11.6	1.85	2.83
4. Zinc sulfate, to give 60 pounds Zn	6	12.4	7.2	5.6	9.1	1.77	2.33	11.8	7.5	7.0	11.8	1.82	2.94
5. Potassium sulfate, to give 120 pounds K	6	12.1	6.4	3.7	6.1	1.23	1.56	11.1	5.9	4.7	9.1	1.44	2.26
6. Boric acid, to give 60 pounds B	6	10.1	6.5	2.8	4.9	.96	1.40	10.1	5.4	2.7	4.8	.89	1.34
7. Soybean meal, to give 400 pounds N	6	12.1	6.9	3.6	5.6	1.08	1.58	12.4	7.1	7.1	9.3	2.08	3.04
8. Treble superphosphate, to give 100 pounds P	4	13.0	7.3	4.7	8.3	1.48	2.18	14.2	9.0	14.9	32.3	4.83	8.72
9. Treble superphosphate, to give 100 pounds P	8	14.0	7.1	6.7	10.5	2.15	2.79	10.2	4.9	2.6	4.5	.80	1.04
10. Treble superphosphate, to give 40 pounds P	6	12.8	6.2	5.0	7.1	1.59	1.94	9.5	5.1	4.1	7.5	1.35	1.94
11. Treble superphosphate, to give 100 pounds P	6	11.8	7.5	3.7	7.4	1.24	2.08	11.0	5.9	3.8	6.9	1.16	1.64
12. Treble superphosphate, to give 500 pounds P	6	12.4	7.0	4.9	8.0	1.51	2.01	11.5	6.1	6.1	11.3	1.85	2.80
13. None	6	13.1	7.7	5.1	8.9	1.72	2.56	11.2	6.9	3.0	5.1	.91	1.46
Minimum mean difference significant at—													
5-percent level		2.30	2.56	3.62	6.72	1.19	1.88	3.78	3.62	8.20	17.6	2.58	4.77
1-percent level		3.14	3.52	4.92	9.20	1.63	2.56	5.16	4.92	11.20	24.1	3.53	6.52

Analysis showed no covariance between height and density. In other words, in this experiment the seedling density was not great enough to affect height growth.

Application of peat resulted in greater density. In September 1938 the height of the seedlings grown in peat-treated soils was greater to a highly significant degree than that of the seedlings grown in soils without peat. When the seedlings were lifted and measured in the spring of 1939, those grown on the peat-treated plots showed a highly significant superiority both in root and top length.

No evidence was found that the mineral salts had caused damage. Perhaps this is due to the fact that they were applied in the fall and were subjected to leaching all winter. It seems likely, however, that the soil absorbed and held most of the soluble cations—although in this light-textured soil the mineral cations probably were diffusely distributed. Injury was less than that on heavier soils reported by some investigators. So far as height growth is concerned, copper, manganese, magnesium, and boron were all ineffective. Density, however, was found in September 1938 to be significantly greater on the plots treated with magnesium and boron than on the untreated plots.

One of the most interesting observations was the effect of soybean meal on density and height. Although application of ammonium nitrate in previous experiments at Chillicothe had resulted in high mortality of seedlings, it had been expected that application of this organic nitrogenous material would not result in injury. However, nitrates rapidly liberated from the soybean meal caused excessive loss of seedlings. The soil surface became grayish green in color from algal growth, and loss from damping-off was very severe.

The effects of the 100-pound application of phosphorus on seedling height and density in September 1938 varied somewhat. On the peat-treated plots phosphorus applied to soil at pH 8 gave poor results both as to density and height. On the no-peat plots, height growth where phosphate was applied at pH 8 was greatly inferior to that where phosphate was applied at pH 4 or 6. Trees on soil at pH 6 not treated for soil reaction responded to phosphorus almost as well as did those on soil at pH 4. This indicates that there was effective phosphorus fixation at pH 8 and none at either pH 4 or pH 6.

In the test of superphosphate at 40, 100, and 500 pounds phosphorus per acre, the 100-pound application did not result very differently from the 40, except in regard to seedling density in September. The 500-pound application resulted in greater seedling height than the 100-pound application on plots not treated with peat, but did not give any superior results elsewhere.

The variations with fertilizer treatment shown by heights recorded early in September of the second year had largely disappeared by the following March, except for plots treated with treble superphosphate at pH 4. Both root and top weights (green and dry) of seedlings on plots phosphate-treated at pH 4 were superior to all others by highly significant differences. The superiority of seedling height on peat-treated soil recorded in September 1938 had not become obscured by differential growth in March 1939. The grand averages for length of tops in September and March, respectively, were 5.9 and 7.8 inches on plots with peat and 4.3 and 6.6 inches on plots without peat.

In the Vallonia as in the Chillicothe nursery, results of the experiment set up in 1937 indicated clearly that acid phosphates stimulated height growth of pines and nitrogen applied at seeding time reduced seedling density. Potassium was probably ineffective.

Root-top ratios for length and for green and dry weights at the time the seedlings were lifted in March of 1939 are presented in table 4. No significant differences according to fertilizer treatment or soil amendment appeared in these ratios. Peat increased height growth significantly root length proportionately.

TABLE 4.—*Average root-top ratios of shortleaf pure seedlings in the spring of 1939 in Vallonia plots, seeded in the fall of 1937*

Fertilizer and rate of application per acre	Soil reaction	Average root-top ratios in spring of 1939 for plots having—					
		Peat			No peat		
		Length	Green weight	Dry weight	Length	Green weight	Dry weight
	pH	*Inches*	*Grams*	*Grams*	*Inches*	*Grams*	*Grams*
1. Copper sulfate, to give 60 pounds Cu	6	1.6	0.57	0.68	1.9	0.60	0.73
2. Manganese sulfate, to give 60 pounds Mn	6	1.6	.56	.67	2.4	.58	.78
3. Magnesium sulfate, to give 60 pounds Mg	6	1.6	.54	.66	1.8	.56	.68
4. Zinc sulfate, to give 60 pounds Zn	6	1.8	.61	.75	1.6	.59	.66
5. Potassium sulfate, to give 120 pounds K	6	2.0	.63	.80	2.0	.59	.75
6. Boric acid, to give 60 pounds B	6	1.8	.60	.73	2.0	.58	.69
7. Soybean meal, to give 400 pounds N	6	1.8	.65	.68	1.8	1.11	.72
8. Treble superphosphate, to give 100 pounds P	4	1.8	.59	.71	1.6	.51	.61
9. Treble superphosphate, to give 100 pounds P	8	2.0	.65	.81	2.1	.58	.77
10. Treble superphosphate, to give 40 pounds P	6	2.1	.69	.81	2.0	.52	.69
11. Treble superphosphate, to give 100 pounds P	6	1.7	.58	.68	1.9	.55	.72
12. Treble superphosphate, to give 500 pounds P	6	1.8	.61	.76	2.0	.55	.69
13. None	6	1.7	.60	.72	1.8	.60	.65
Average		1.8	.61	.73	1.9	.61	.70
Minimum mean difference (for values for single treatments) significant [1] at—							
5-percent level		1.8	.34	.20	1.8	.34	.20
1-percent level		2.4	.46	.26	2.4	.46	.26

[1] Values were calculated on the basis of complete data, not of averages.

1938 EXPERIMENTS

Since in 1937 only one form of fertilizer was used for each nutrient element, a question arose as to the probable effectiveness of other carriers. Further tests of peat were needed because response to peat had not been the same on the Vallonia as on the Chillicothe nursery soil. Further study of the influence of soil reaction, also, was needed.

In the spring of 1938 a fertilizer experiment was started at each of the three nurseries to test the effectiveness of five kinds of phosphatic material—treble superphosphate, rock phosphate, iron phosphate, aluminum phosphate, and liquid phosphoric acid. Aluminum phosphate was applied at Chillicothe only and iron phosphate at Vallonia only. Tests were made also of four kinds of nitrogenous

material—ammonium sulphate, sodium nitrate, dried blood, and soybean meal—and of three kinds of potassic material—potassium sulphate, tobacco stems, and green sand marl. Each of the three series of materials containing phosphorus, nitrogen, and potassium, respectively, was randomized completely in the nursery beds. In addition, peat was applied on randomly selected phosphate and nitrogen plots at Chillicothe and Vallonia. Soil reaction was varied by applying finely ground limestone or sulfuric acid. All tests were replicated. The experimental design provided an opportunity for evaluating each fertilizing material individually, without any attempt to study nutrient combinations.

TABLE 5.—*Average density and height in September 1938, of pitch pine seedlings treated with different fertilizer materials and soil amendments, in plots at Chillicothe, seeded in the spring of 1938*

Fertilizer and rate of application per acre	Average seedling measurements in September 1938.	
	Density per square foot	Height
	Number	*Inches*
Phosphorus:		
Aluminum phosphate, to give 400 pounds P	9	2.90
Rock phosphate, to give 400 pounds P	11	1.57
Treble superphosphate, to give 100 pounds P	10	3.22
Liquid phosphoric acid, to give 100 pounds P	9	2.79
None	11	1.72
Minimum mean difference significant at—		
5-percent level	1.9	.37
1-percent level	2.54	.50
Nitrogen:		
Soybean meal, to give 100 pounds N	7	1.50
Dried blood, to give 100 pounds N	4	1.40
Ammonium sulfate, to give 100 pounds N	2	1.36
Sodium nitrate, to give 100 pounds N	8	1.60
None	9	1.58
Minimum mean difference significant at—		
5-percent level	3.6	.89
1-percent level	5.2	.57
Potassium:		
Potassium sulfate, to give 100 pounds K	12	1.42
Tobacco stems, to give 100 pounds K	7	1.20
Greensand marl, to give 500 pounds K	9	1.40
None	5	1.08
Minimum mean difference significant at—		
5-percent level	4.1	.45
1-percent level	6.0	.66
Lime on phosphate plots (ground limestone):		
2 tons	8	2.26
None	12	2.62
Minimum mean difference significant at—		
5-percent level	1.2	.20
1-percent level	1.6	.32
Peat on phosphate plots:		
4 tons	10	2.64
None	10	2.24
Minimum mean difference significant at—		
5-percent level	1.2	.20
1-percent level	1.6	.32

The materials were applied at the following rates per acre: Rock phosphate, iron phosphate, and aluminum phosphate, 400 pounds phosphorus; nitrogenous substances, 100 pounds nitrogen; potassium

sulfate and tobacco stems, 100 pounds potassium; greensand marl, approximately 500 pounds potassium; peat, 4 tons; and calcium carbonate, 2 tons. At Chillicothe and at Vallonia, soil reaction was adjusted to the two pH levels 4 and 7 (or slightly higher) by use of sulfuric acid and limewater, respectively. All soil amendments and fertilizer applications were made at seeding time. Pitch pine seed was used at Chillicothe and Vallonia, shortleaf pine seed at Licking.

CHILLICOTHE NURSERY

Seedling counts and height measurements were taken in the Chillicothe nursery early in September of 1938, with the results presented in table 5. Height growth on the plots treated with rock phosphate did not differ significantly from that on the check plots, but liquid phosphoric acid, aluminum phosphate, and treble superphosphate gave significantly greater seedling heights. Aluminum phosphate is ordinarily considered unavailable, but here, probably because of the relatively high reaction of the soil, it was as effective as liquid phosphoric acid. Liquid phosphoric acid and aluminum phosphate caused lower seedling density; this is attributed to natural thinning induced by increased growth. Density was drastically reduced by ammonium sulfate and dried blood. Dried blood is not immediately available, but nitrifies rapidly in warm moist soil. Injury from dried blood was not considered "burning," but was thought to have resulted from attacks of damping-off fungi that had been stimulated by the rapid evolution of nitrates. Peat increased seedling height where used with phosphorus, but did not do so where used with nitrogen or potassium. Lime caused lower density on phosphate plots.

Seedling density on the plots treated with potassium sulfate was greater than that on the check plots.

VALLONIA NURSERY

At the Vallonia nursery, seedling counts were taken in June 1938 and both density and height were measured in September of that year, with the results presented in table 6. In the spring of 1939 the seedlings on the sample strips were lifted and washed, measured, and weighed.

In the June counts no significant effects on seedling density were noted on the phosphate plots. A highly significant difference in density appeared between the nitrogen plots and the check plots, and between the limed and the unlimed plots. Potassium fertilizers had little or no effect on density. When counts and height measurements were made in September, no significant differences in either density or height were found that could be attributed to application of phosphorous, nitrogen, or potassium. When the seedlings were lifted in the spring of 1939, no significant differences in root length, top length, or root or top green or dry weight resulting from nitrogen or potassium additions were noted; but highly significant differences from check-plot values in length of both roots and tops and in green and dry weights were recorded for all plots treated with prosphatic materials other than rock phosphate (table 7). On the nitrogen-treated plots lime decreased top length, but did not affect root length or green or dry weight of roots or tops. Root-top length ratios for seedlings grown on nitrogen plots are given in table 8. On the phosphorus-

treated plots lime did not affect root or top length; it did reduce the green and dry weights of both roots and tops. Peat had no significant effects.

TABLE 6.—*Average density in June and September 1938 and average height in September 1938 of pitch pine seedlings treated with different fertilizer materials and soil amendments, in plots at Vallonia, seeded in the spring of 1938*

Fertilizer and rate of application per acre	Average seedling density per square foot		Average seedling height in September 1938
	June 1938	September 1938	
Phosphorus:	*Number*	*Number*	*Inches*
Iron phosphate, to give 400 pounds P	65	11	4.1
Rock phosphate, to give 400 pounds P	74	16	3.9
Treble superphosphate, to give 100 pounds P	70	16	3.8
Liquid phosphoric acid, to give 100 pounds P	65	12	4.0
None	79	12	4.0
Minimum mean difference significant at—			
5-percent level	19	6	.7
1-percent level	25	9	.9
Nitrogen:			
Soybean meal, to give 100 pounds N	8	4.0	2.6
Dried blood, to give 100 pounds N	12	2.6	2.4
Ammonium sulfate, to give 100 pounds N	32	3.6	2.1
Sodium nitrate, to give 100 pounds N	20	4.6	1.8
None	64	2.9	2.0
Minimum mean difference significant at—			
5-percent level	13	8	3.4
1-percent level	18	11	4.6
Potassium:			
Potassium sulfate, to give 100 pounds K	50	16	1.8
Tobacco stems, to give 100 pounds K	61	8	1.8
Greensand marl, to give 500 pounds K	57	13	2.0
None	65	5	1.6
Minimum mean difference significant at—			
5-percent level	52	19	.6
1-percent level	75	27	.8
Lime [1] (ground limestone):			
2 tons	19	2.8	3.6
None	39	4.3	4.0
Minimum mean difference significant at—			
5-percent level	12	4.0	.5
1-percent level	16	5.0	.6

[1] Values presented are for plots on which phosphorus was applied.

TABLE 7.—*Average measurements in the spring of 1939, of pitch pine seedlings treated with different phosphatic materials, in plots at Vallonia, seeded in the spring of 1938*

Fertilizer and rate of application per acre	Average seedling measurements in spring of 1939					
	Length		Green weight		Dry weight	
	Root	Top	Root	Top	Root	Top
	Inches	*Inches*	*Grams*	*Grams*	*Grams*	*Grams*
Iron phosphate, to give 400 pounds P	11.4	6.0	2.72	4.74	0.80	1.40
Rock phosphate, to give 400 pounds P	10.2	5.0	1.70	2.96	.46	.82
Treble superphosphate, to give 100 pounds P	11.1	6.1	2.61	4.78	.73	1.35
Liquid phosphoric acid, to give 100 pounds P	11.6	6.1	2.61	5.08	.86	1.46
None	10.4	5.2	1.80	3.06	.46	.86
Minimum mean difference significant at—						
5-percent level	.6	.6	.49	.98	.14	.26
1-percent level	.7	.8	.65	1.31	.19	.35

TABLE 8.—*Average root-top length ratios in the spring of 1939 of pitch pine seedlings treated with nitrogen, in plots at Vallonia, seeded in the spring of 1938*

Fertilizer and rate of application per acre	Average root-top length ratios in spring of 1939 for plots having—	
	Lime	No lime
Soybean meal, to give 100 pounds N	2.59	1.91
Dried blood, to give 100 pounds N	2.47	2.25
Ammonium sulfate, to give 100 pounds N	2.26	2.31
Sodium nitrate, to give 100 pounds N	2.65	1.88
None	2.14	2.13
Minimum mean difference significant at—		
5-percent level	0.58	
1-percent level	.79	

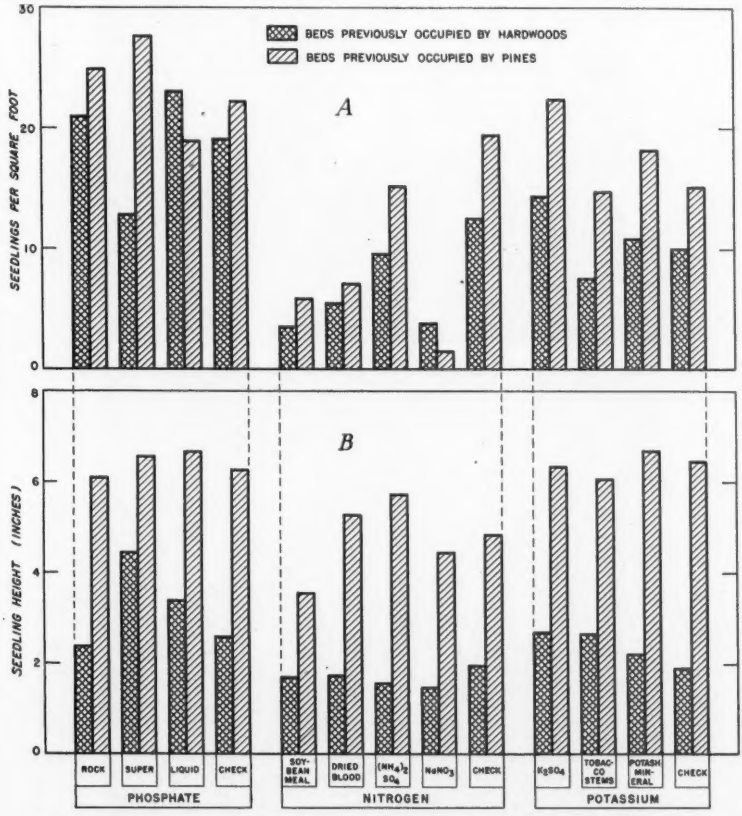

FIGURE 3.—Density (*A*) and height (*B*) in October 1938 of shortleaf pine seedlings on uninoculated and inoculated Licking plots, seeded in the spring of that year, treated with three kinds of phosphatic, four kinds of nitrogenous, and three kinds of potassic fertilizers. The fertilizers used, and rates of application per acre, were as follows: Rock phosphate, to give 400 pounds P; treble superphosphate, to give 100 pounds P; liquid phosphoric acid, to give 100 pounds P; soybean meal, to give 600 pounds N; dried blood, to give 100 pounds N; ammonium sulfate, to give 100 pounds N; sodium nitrate, to give 100 pounds N; potassium sulfate, to give 100 pounds K; tobacco stems, to give 100 pounds K; and potash mineral, to give 400 pounds K.

FIGURE 4.—Shortleaf pine seedbeds in Licking nursery of which one (*A*) had previously been in hardwoods and the other (*B*) had previously been in pines. These beds were seeded the same day and received identical treatment. The taller of the seedlings shown in *A* are on phosphate-treated plots.

LICKING NURSERY

In the Licking nursery the 1938 experiment differed from those of the same year at Chillicothe and Vallonia in that soil reaction was not altered, shortleaf pine was used instead of pitch pine, and plots were established by identical methods on soil previously in hardwoods and on soil previously in pines. The plot series on hardwood and pine soil will be referred to here as the hardwood-soil series and the pine-soil series. In October 1938 the seedlings on the sample strips were counted and measured for height. In the spring of 1939 they were lifted and washed, measured, and weighed. The results of the fall and the spring measurements are given in tables 9 and 10, and those of the fall measurements are presented in figure 3.

TABLE 9.—*Average measurements in the fall of 1938 of shortleaf pine seedlings treated with fertilizer materials and soil amendments, in plots at Licking, seeded in the spring of 1938*

Fertilizer and rate of application per acre	Average seedling measurements in October 1938			
	Hardwood-soil series		Pine-soil series	
	Density per square foot	Height	Density per square foot	Height
	Number	*Inches*	*Number*	*Inches*
Phosphorus:				
Rock phosphate, to give 400 pounds P	20.8	2.4	24.8	6.1
Treble superphosphate, to give 100 pounds P	12.9	4.5	27.6	6.6
Liquid phosphoric acid, to give 100 pounds P	23.0	3.4	19.1	6.7
None	19.2	2.6	22.1	6.3
Minimum mean difference significant at—				
5-percent level	5.1	.8	6.8	.5
1-percent level	7.1	1.2	9.4	.8
Nitrogen:				
Soybean meal, to give 600 pounds N	3.6	1.7	5.8	4.9
Dried blood, to give 100 pounds N	5.3	1.8	7.2	4.5
Ammonium sulfate, to give 100 pounds N	9.6	1.6	15.2	5.8
Sodium nitrate, to give 100 pounds N	3.9	1.5	1.6	4.5
None	12.6	2.0	19.6	4.9
Minimum mean difference significant at—				
5-percent level	6.5	.25	2.8	1.1
1-percent level	8.9	.34	3.9	1.5
Potassium:				
Potassium sulfate, to give 100 pounds K	14.5	2.7	22.4	6.4
Tobacco stems, to give 100 pounds K	7.6	2.7	14.8	6.1
Potash mineral, to give 400 pounds K	10.8	2.2	18.2	6.7
None	10.0	1.9	15.2	6.5
Minimum mean difference significant at—				
5-percent level	6.6	.7	4.7	1.4
1-percent level	10.1	1.1	7.1	2.1
Peat on phosphorus plots:				
4 tons	20.0	3.5	26.0	6.6
None	19.0	2.9	21.0	6.2
Minimum mean difference significant at—				
5-percent level	4.0	.6	4.8	.39
1-percent level	5.0	.8	6.7	.54
Peat on nitrogen plots:				
4 tons	7.0	1.8	13.0	5.0
None	7.0	1.7	7.0	4.8
Minimum mean difference significant at—				
5-percent level	.41	.16	1.8	.7
1-percent level	.57	.22	2.4	.9

The outstanding observation in this 1938 Licking experiment was the superiority of seedlings grown on soil previously in pines as compared with those grown on other soil (fig. 4). When the seedlings

on the sample strips were lifted it was found that the roots of those grown after pines had many short, fleshy gray rootlets, but that the roots of those grown in soil not previously occupied by pines were brown and wiry, without short fleshy rootlets.

TABLE 10.—*Average measurements in the spring of 1939, of shortleaf pine seedlings treated with fertilizer materials and soil amendments, in plots at Licking, seeded in the spring of 1938*

Fertilizer and rate of application per acre	Average seedling measurements in spring of 1939									
	Hardwood-soil series				Pine-soil series					
	Length		Green weight		Length		Green weight		Air-dry weight	
	Root	Top	Root	Top	Root	Top	Root	Top	Root	Top
Phosphorus:	*Inches*	*Inches*	*Grams*	*Grams*	*Inches*	*Inches*	*Grams*	*Grams*	*Grams*	*Grams*
Rock phosphate, to give 400 pounds P	8.60	2.88	0.75	1.25	10.41	6.35	3.70	5.70	1.38	1.94
Treble superphosphate, to give 100 pounds P	10.85	5.50	2.75	4.48	10.07	6.90	3.66	5.88	1.40	2.06
Liquid phosphoric acid, to give 100 pounds P	9.71	4.02	1.42	2.16	10.58	7.00	4.40	7.60	1.55	2.56
None	8.55	2.94	1.00	1.60	10.80	7.10	4.32	6.87	1.63	2.36
Minimum mean difference significant at—										
5-percent level	.85	.94	.55	1.26	1.03	.99	.73	1.37	.36	.43
1-percent level	1.18	1.30	.76	1.74	1.43	1.38	1.02	1.90	.49	.60
Nitrogen:										
Soybean meal, to give 100 pounds N					9.93	5.60	4.35	5.85	1.50	1.98
Dried blood, to give 100 pounds N					10.20	5.58	4.52	6.20	1.70	2.21
Ammonium sulfate, to give 100 pounds N					10.47	5.67	4.20	5.98	1.55	2.08
Sodium nitrate, to give 100 pounds N					10.90	6.04	4.88	6.68	1.80	2.27
None					10.20	5.50	3.83	5.42	1.48	1.94
Minimum mean difference significant at—										
5-percent level					1.13	.71	1.09	1.50	.37	.51
1-percent level					1.55	.97	1.50	2.06	.51	.70
Potassium:										
Potassium sulfate, to give 100 pounds K	7.16	2.46	1.08	1.42	10.30	6.23	3.50	5.30	1.33	1.82
Tobacco stems, to give 100 pounds K	8.00	3.23	1.51	2.04	11.10	6.23	3.96	5.80	1.50	1.97
Greensand marl, to give 100 pounds K	6.07	2.73	.97	1.42	10.23	6.47	3.57	6.00	1.52	2.04
None	7.03	2.80	1.22	1.66	10.60	6.17	3.93	6.33	1.48	2.15
Minimum mean difference significant at—										
5-percent level	2.04	.58	.93	.95	.75	.72	.83	1.82	.51	.82
1-percent level	2.91	.82	1.41	1.42	1.13	1.09	1.14	2.51	.78	1.25
Peat:[1]										
4 tons			1.61	2.50						
None			1.34	2.25						
Minimum mean difference significant at—										
5-percent level			.39	.89						
1-percent level			.54	1.23						

[1] Values presented are for plots on which phosphorus was applied.

Measurements in the fall of the first year showed that superphosphate and liquid phosphoric acid had increased height growth in seedlings on hardwood soil. Lower seedling density was associated

with superphosphate treatment. Phosphate did not influence seedling height on pine soil. Retarded height growth and lower seedling density were associated with the soybean meal, ammonium sulfate, and sodium nitrate treatments on hardwood soil. On pine soil none of the nitrogenous fertilizer treatments affected seedling height, but all of them reduced density. Only in one instance did potassium affect seedlings materially in this experiment; higher density was associated with potassium sulfate on pine soil.

Where phosphorus was applied in 1938, peat had little or no effect on either density or height on hardwood soil, but increased density and height on pine soil. In the nitrogen tests on hardwood soil peat did not increase either density or height, but in those on pine soil it increased density.

When lifted in the spring of 1939, the seedlings grown on some hardwood-soil plots showed beneficial effects from the application of phosphates. Those from plots treated with liquid phosphoric acid were superior in top and root length and top weight, and those from plots treated with treble superphosphate were superior in both length and weight of tops and roots. The seedlings on hardwood-soil plots treated with nitrogenous fertilizers failed completely in 1939; not enough remained for an adequate sample. Similar mortality did not occur on pine-soil plots. There was no evidence that the seedlings on pine soil had been affected in any way by any of the fertilizers applied.

1939 EXPERIMENTS

When on completion of the 1938 experiments it became evident that phosphorus was the most effective fertilizing element, and that nitrogen in any form applied at seeding time greatly lowered pine seedling density, it was decided to test application of nitrogenous fertilizers after seeding. A combination experiment was designed for three levels each of phosphorus, nitrogen, and potassium, applied as treble superphosphate, ammonium sulfate, and potassium sulfate. These levels, with the times of application, were: Phosphorus—none, 40 pounds at seeding time, and 100 pounds at seeding time; nitrogen—none, 25 pounds 2 weeks after seedling emergence, and 25 pounds 4 weeks after emergence; potassium—none, 25 pounds 2 weeks after emergence, and 25 pounds 4 weeks after emergence. Three experimental blocks were established, each containing twenty-seven 3- by 4-foot plots representing the 27 combinations of the 3 mineral nutrients at 3 levels each, completely randomized.

In addition to this test of nitrogen and potassium, a further test was made of rock phosphate and greensand marl on soils differing in reaction. It was thought possible that failure to get results with these materials previously had been caused by unfavorable soil reaction. On one-third of the plots receiving rock phosphate or greensand marl, calcium carbonate was added in excess in order to raise the reaction to the neutral point. On another third, sufficient dilute sulphuric acid was sprinkled to reduce the soil reaction to about pH 4. On the remainder the soil reaction, approximately pH 6, was not altered.

This experimental design was used at each of the three nurseries, with shortleaf pine. At Chillicothe more than half the seedlings

died en bloc from causes independent of treatment; therefore this part of the experiment was abandoned.

In the spring of 1940, at Vallonia and Licking, the seedlings on the sample strips were lifted, washed, measured, and weighed green. The average root and top lengths and weights for the phosphorus-nitrogen-potassium experiment are given in table 11 and those for the rock phosphate-greensand marl experiment in table 12.

Association of greater lengths of roots and tops with phosphate treatment was consistent throughout the 1939 experiments. It should be noted that 100 pounds of phosphorus per acre gave better results than 40 pounds. Nitrogen increased root and top length at Vallonia and reduced top length at Licking. It increased root and top weight at Vallonia but not at Licking. At Vallonia the seedlings on plots treated with nitrogenous salts 2 weeks after seedling emergence were superior in top length and in root and top weights to those on plots not so treated; and seedlings on plots similarly treated 4 weeks after emergence were superior in all measurements. At Licking, ammonium sulfate applied 2 weeks after seedling emergence had no effect on root or top length or weight and the same application 4 weeks after emergence was ineffective, except that it reduced top length. In no case did potassium influence growth.

Some beneficial results from the application of rock phosphate were indicated, but they did not rise to significant levels. No beneficial results could be found from the use of greensand marl on soil at pH 4, 6, or 7, in either nursery. A combination of rock phosphate and greensand marl gave a highly significant increase in top weight at Licking in soil at pH 6.

TABLE 11.—*Average measurements in the spring of 1940, of seedlings treated with phosphorus, nitrogen, and potassium, in plots at Vallonia and Licking, seeded in the spring of 1939*

Fertilizer treatment [1]	Vallonia					Licking				
	Average density per square foot	Average length		Average green weight		Average density per square foot	Average length		Average green weight	
		Root	Top	Root	Top		Root	Top	Root	Top
	Number	*Inches*	*Inches*	*Grams*	*Grams*	*Number*	*Inches*	*Inches*	*Grams*	*Grams*
Phosphorus:										
P_0	36	10.5	5.6	1.6	3.5	17	12.2	6.5	3.7	6.8
P_1	33	10.7	5.8	1.7	3.6	13	12.2	6.6	4.1	7.2
P_2	29	11.1	6.2	2.0	4.2	11	12.6	7.0	4.6	8.6
Nitrogen:										
N_0	34	10.5	5.4	1.6	3.2	13	12.6	6.9	4.2	7.8
N_1	32	10.8	6.2	1.9	4.2	13	12.4	6.8	4.3	7.7
N_2	33	10.9	6.0	1.9	4.0	13	12.1	6.4	4.0	7.1
Potassium:										
K_0	33	10.8	5.8	1.7	3.5	13	12.7	6.8	4.3	7.8
K_1	32	10.9	6.0	1.8	4.0	13	12.1	6.7	4.1	7.6
K_2	33	10.6	5.8	1.8	3.8	13	12.4	6.6	4.0	7.2
Minimum mean difference significant at—										
5-percent level	4.18	.4	.4	.2	.5	1.70	.6	.4	.4	.8
1-percent level	5.58	.5	.5	.3	.6	2.26	.8	.5	.6	1.1

[1] Key:

Phosphorus—
P_0=None.
P_1=40 pounds at seeding time.
P_2=100 pounds at seeding time.

Nitrogen and potassium—
N_0 or K_0=None.
N_1 or K_1=25 pounds per acre 2 weeks after seedling emergence.
N_2 or K_2=25 pounds per acre 4 weeks after seedling emergence.

TABLE 12.—*Average measurements in the spring of 1940, of seedlings treated with rock phosphate, greensand marl, or a combination of the two, in plots at Vallonia and Licking, seeded in the spring of 1939*

Nursery and potassium treatment [1]	Length of seedlings grown in soil having a reaction of—						Green weight of seedlings grown in soil having a reaction of—					
	pH 4		pH 6		pH 7+		pH 4		pH 6		pH 7+	
	Root	Top	Root	Top	Root	Top	Root	Top	Root	Top	Root	Top
Vallonia:	*In.*	*In.*	*In.*	*In.*	*In.*	*In.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
RM	10.7	4.6	11.1	5.0	10.1	4.7	1.8	3.3	1.2	2.0	1.5	2.5
R	11.6	5.2	10.9	4.4	10.8	4.6	1.5	2.8	1.7	2.8	1.9	3.3
M	10.9	5.0	10.2	3.9	10.1	4.0	2.1	3.6	1.4	2.3	2.2	3.9
None	10.7	4.6	11.3	4.4	10.6	4.2	1.9	3.4	1.4	2.5	1.9	3.2
Minimum mean difference significant at—												
5-percent level	1.00	1.27	1.00	1.27	1.00	1.27	.76	1.70	.76	1.70	.76	1.70
1 percent level	1.36	1.73	1.36	1.73	1.36	1.73	1.04	2.31	1.04	2.31	1.04	2.31
Licking:												
RM	13.2	6.4	13.6	7.1	14.0	6.4	3.8	6.6	5.1	10.4	5.2	8.7
R	12.9	6.7	12.8	7.1	13.4	5.9	4.1	7.2	3.4	7.4	3.8	8.1
M	13.1	6.1	12.8	6.1	14.3	6.1	3.8	6.3	4.4	7.5	4.9	7.8
None	13.5	6.1	13.0	6.4	13.3	6.5	3.8	6.7	3.9	6.4	4.5	7.9
Minimum mean difference significant at—												
5-percent level	1.66	1.08	1.66	1.08	1.66	1.08	1.56	2.38	1.56	2.38	1.56	2.38
1-percent level	2.26	1.47	2.26	1.47	2.26	1.47	2.11	3.24	2.11	3.24	2.11	3.24

[1] Key: R=Rock phosphate, 4 tons per acre.
M=Greensand marl, 4 tons per acre.
RM=Combination of R and M.

Density was not affected by either nitrogen or potassium, but was reduced by phosphorus (table 11). At the Vallonia nursery, seedlings per square foot averaged 36, 33, and 29, respectively, for no phosphorus, 40 pounds phosphorus, and 100 pounds phosphorus per acre; and at the Licking nursery they averaged 17, 13, and 11, respectively, for the same applications. Although density varied inversely with quantity of phosphorus applied, height varied directly with it.

DISCUSSION

The requirement of light texture for easy workability places certain handicaps on the maintenance of nursery soils. Light-textured, well-drained soils have high oxidation rates, hence their organic-matter content usually is not only low at the time of nursery establishment but is likely to require the addition of large quantities of organic matter for its maintenance. It is doubtful whether the organic-matter content of very light soils can be permanently maintained by the use of cover crops, to say nothing of increasing it in this way.

Well disintegrated peat is probably the best means of adding organic matter to nursery soil. It resists oxidation and ameliorates soil structure. It also serves as a medium for holding acid, where acid must be applied to reduce the alkalinity of the soil. Peat helps to hold moisture in the upper soil, and insulates it against rapid evaporation. The beneficial effects of peat added to light nursery soils are indicated in the results of the experiments discussed here. Peat was particularly effective in the 1937 Vallonia tests, increasing both density and height of shortleaf pine seedlings. In the 1938 tests

it increased height of pitch pine on phosphate-treated plots at Chillicothe, increased density and height of shortleaf pine on phosphate-treated plots previously in pines at Licking, and increased density on nitrogen-treated plots previously in pines at Licking. Its effects were not entirely consistent, for in the 1938 tests it failed to cause any increase in seedling height or density at Vallonia, where it had been very effective in the 1937 tests. Perhaps this inconsistency was due to the more efficient sprinkling in 1938, which may have canceled any possible advantage from the water-holding capacity of the peat.

The light texture necessary for workability in a forest nursery soil increases the difficulty of maintaining an optimum nitrogen supply because rapid aeration causes rapid oxidation and evolution of nitrates from organic debris, and free drainage quickly removes soluble nitrates. Nitrification of large quantities of organic matter is injurious to pine seedlings during the germination and emergence period. All the forms of nitrogenous fertilizer used in these experiments reduced seedling density where applied at seeding time. Soluble nitrogenous fertilizers were most beneficial where applied about 2 weeks after seedling emergence. Soluble mineral nutrients of any kind are injurious unless applied in moderate quantities and quickly washed into the soil. Soybean meal in quantities smaller than those used in this study might have given beneficial results, but it seems probable that nitrates characteristically have an undesirable effect on pine seedlings in the early stages. Possibly soybean meal might be used to advantage as a surface dressing 2 weeks after seedling emergence. At that time it would be less likely to "burn" the seedlings or to increase damping-off through stimulation of soil fungi.

The fact that no form of potassic fertilizer increased pine seedling height on the soils studied does not mean that potassium is never beneficial to pine seedlings. The soils studied evidently were well supplied with potassium; this, however, is not true of some other soils in which pine seedlings are being or might be grown. Where potassium is needed it should be applied only in moderate quantities and after seedling emergence.

Pine seedlings have often been observed to fail even on soil containing ample mineral nutrients. The relation between mycorrhizae and seedling nutrition is as yet largely unexplained. Solubility of phosphates is known to vary with soil reaction. In acid soils well supplied with iron and aluminum phosphates, it is distinctly possible that fungi make these phosphates available by raising the soil reaction locally. On one soil not inoculated with mycorrhizal fungi, application of easily soluble phosphates increased thrift of pine seedlings. Application of such phosphates, however, did not raise this soil's production of pine seedlings to that of the same soil on an area where pines had been grown the previous year. A lag in development of some sort of biotic balance has frequently been observed in nursery soil newly converted from field crops to conifer seedlings. Pine seedlings having mycorrhizal roots are usually thrifty.

No specific study of mycorrhizae was made in this investigation. Observations are reported only as indications of the need for more facts regarding the interrelations of mineral nutrients, mycorrhizae, and pines.

Apparently the most satisfactory form of phosphatic fertilizer for pine nursery soil is treble superphosphate. Results of this study indi-

cate that in view of effectiveness and cost, 500 pounds per acre is the maximum quantity of treble superphosphate that should be applied. In some cases this rate may prove excessive, stimulating growth to such an extent that tops and roots must be pruned.

Ample justification exists for expecting beneficial results from the application of rock phosphate, although inconclusive results were obtained in these tests. On many plots treated with it a trend toward greater length of roots and height of tops was observed. In the acid soil that is necessary for pine seedlings, rock phosphate should be available. The light texture and organic-matter deficiency of the soils in which these experiments were made are unfavorable to liberation of phosphoric acid from rock phosphate. As the organic-matter supply in the soil increases, yearly additions of rock phosphate should in time augment the total supply to a point at which the annual absorption of phosphorus by the pine seedling crop is currently replaced. Continued addition of large quantities of superphosphate not adjusted to current absorption and loss may result in soil saturation and seedling injury. Chlorosis has been known to result from iron deficiency caused by excess of phosphorus in the soil.

SUMMARY

Exploratory research directed toward better selection and soil management of nursery sites for pines in the Central States was conducted during a 5-year period beginning in 1936. Three Forest Service nurseries were used, those at Chillicothe, Ohio, Vallonia, Ind., and Licking, Mo., which had been established in 1934–36 on land previously used for agriculture. The nurseries at Chillicothe and Vallonia had sandy soils that were alkaline and subject to crusting as a result of concentration of calcium salts from evaporation of soil water or sprinkling water; the Licking nursery had a somewhat heavier silt loam soil that was slightly acid and consequently not subject to this form of crusting. Efforts were made to determine mineral nutrient requirements and deficiencies, and to devise effective methods of amending and fertilizing soils and adjusting soil acidity. The tree species used were shortleaf pine and pitch pine.

Fertilizing tests were started at Chillicothe in the spring of 1937 involving the application of phosphorus, nitrogen, potassium, peat, lime, and combinations thereof. At Vallonia in the fall of 1937 tests were started with 13 treatments including some of the minor elements, soybean meal, phosphorus at three rates of application and at three levels of soil reaction, and potassium. At the three nurseries, tests were started in the spring of 1938 with five phosphatic, four nitrogenous, and three potassic materials together with lime and peat. The 1938 tests at the Licking nursery included a comparison between soil previously occupied by pines and soil previously occupied by hardwoods. At the completion of these experiments tests were set up, in 1939, in which treble superphosphate was applied at seeding time at two different rates per acre and soluble nitrates and soluble potassic salts were applied 2 weeks and 4 weeks, respectively, after seedling emergence. Rock phosphate and greensand marl were further tested in 1939 in conjunction with adjustment of soil reaction by excessive addition of calcium carbonate and by use of dilute sulphuric acid. Results were judged on the basis of seedling density,

height in the first and the second year, and root and top lengths and weights in the second and the third year.

The experiments resulted as follows:

Biotic balance was lacking in soil at the Licking nursery where pines were grown after hardwoods. Where treble superphosphate was applied, increased height growth indicated that it partially compensated for this deficiency. Pines on nearby plots that had been occupied by pines the previous year were uniformly thrifty regardless of whether treble superphosphate had been applied.

Acid-treated peat incorporated in surface soil corrected alkalinity resulting from concentration of calcium salts. In general, peat had a beneficial effect on both density and height.

Increase in soil alkalinity brought about by application of ground limestone resulted in lower seedling density.

Liquid phosphoric acid, treble superphosphate, and iron phosphate increased height growth, root length, and root weight. Aluminum phosphate increased height growth. Height-growth response to these phosphatic fertilizers was more pronounced in soil at pH 4 or 6 than in soil at pH 8. Seedling density varied inversely with quantity of phosphorus applied in these forms. Rock phosphate, applied at the rate of 400 pounds P per acre to soil at pH 4, 6, or 8, did not significantly influence growth or density, although on many plots treated with it a trend toward greater length of roots and height of tops was observed.

Magnesium, potassium, and boron each brought about significantly greater seedling density on Vallonia nursery soil, classifiable as Princeton sandy loam, fall-seeded to shortleaf pine. Potassium sulfate increased density of pitch pine and shortleaf pine seedlings, at Chillicothe and Licking, respectively, measured in the fall of 1938. Copper, manganese, magnesium, zinc, and boron applied at Vallonia all failed under the conditions just mentioned to cause any growth response.

Ammonium sulfate applied 2 and 4 weeks after seedling emergence at the rate of 25 pounds of nitrogen per acre had a favorable effect on length of tops and on weight of roots and tops of shortleaf pine seedlings on Princeton sandy loam. On Huntington silt loam, ammonium sulfate applied 2 weeks after seedling emergence had no perceptible effect on seedling height growth, but the same application 4 weeks after seedling emergence lessened height growth.

No form of potassium tested stimulated height growth of pine seedlings in any of the three nursery soils.

Nitrogen applied at seeding time, in all the forms tested, greatly lowered pine seedling density.

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